ISOLATION AND BIOASSAY OF *STRIGA HERMONTHICA* SEED GERMINATION STIMULANTS FROM NON-HOST CROPS AND FIELD TESTING FOR CONTROL EFFICACY

By

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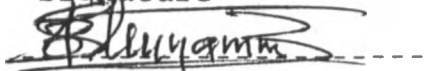and Veterinary Sciences, of the <u>University of Nairobi</u>

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DECLARATION

I declare that the work presented in this thesis is original and has not been presented in any other university for the purpose of awarding a degree.

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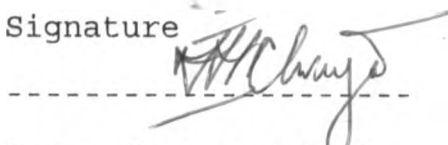
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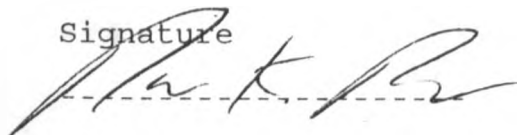
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DEDICATION

TO MY WIFE JANE L. A. SAFARY

MY CHILDREN ELVIS, LAVEEN AND EDMUND

AND

MY LATE FATHER BENEDICTO ARIGA SOMBE

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

TITLE: ISOLATION AND BIOASSAY OF *STRIGA HERMONTHICA*   PAGE

SEED GERMINATION STIMULANTS FROM NON-HOST

CROPS AND FIELD TESTING FOR CONTROL EFFICACY

CHAPTER 1: EFFECTS OF NON-HOST EXTRACTS AND STIMULANTS
   ON GERMINATION OF *STRIGA HERMONTHICA* SEEDS

LIST OF TABLES

CHAPTER 4

LIST OF FIGURES

LIST OF APPENDICES   **PAGE**

GENERAL ABSTRACT

Striga hermonthica (Del.) Benth. is a severe parasite on maize and a limiting factor in achieving optimum maize yields in infested areas in Africa. The parasite seeds are stimulated to germinate by root exudates from host and non-host plants as well as by synthetic germination stimulants. Objectives of these experiments were to determine laboratory, screenhouse and field conditions for inducing maximum germination of *S. hermonthica* seeds with extracts from cultivars of cowpea (*Vigna unguiculata* (L) Walp.), cotton (*Gossypium* spp.) and soybean (*Glycine max.* L.) plants, their residues and a synthetic germination stimulant, GR 24.

Extracts of cowpea roots and shoots stimulated about 30 to 40% as much germination as 10^{-2} mgL^{-1} GR 24. No differences were observed among cowpea cultivars in germination percent of *S. hermonthica* seeds induced by the aqueous extracts. The population from Mokwa responded most to the extracts. Increasing concentrations of both whole cowpea plant extracts and GR 24 resulted in increased *S. hermonthica* seed germination upto 6.3mg plant tissue mL^{-1} of water and 10^{-2} mgL^{-1} respectively, beyond which germination decreased. Higher germination was recorded when incubation time of *S. hermonthica* seeds with GR 24 at 30°C was increased from 24 to 48 hours in microtiter wells. Storing plant extracts at 7°C for 20 days did not reduce potency.

Germination of *S. hermonthica* seeds by cotton and cowpea plant parts were dependent on the source of the stimulant, weight of plant part and the distance of the parasite seeds from the source of the stimulant. Germination of *S. hermonthica* seeds decreased with increase in weight of plant part and period of time each cultivar was grown. Cotton roots contained the most effective *S. hermonthica* seed germination stimulant among the plant materials tested. With both *S. hermonthica* and *S. aspera*, mixtures of cotton extracts from different plant parts depressed germination of the seeds below the average induced by individual extracts in the mixture. Some extracts and mixtures were as effective as 10^{-2} mgL-1 of GR 24.

DCM soluble extracts of cotton and cowpea plant parts of different ages separated by thin layer chromatography showed that the extracts contained several similar compounds as shown by position of relative fronts. DCM extracts of *S. hermonthica* seeds contained similar compounds. Germination of *S. hermonthica* seeds by DCM soluble extracts of cotton and cowpea roots increased with increasing concentration from 0.1 to 0.5 % and 0.1 to 1.0 % for cotton and cowpea respectively and then declined with further increase in concentration.

Soybean extracts from TGX-1674-1F was as effective as 10^{-3} mgL^{-1} GR 24 in inducing germination of *S. hermonthica* seeds collected from sorghum in Zaria, Mokwa and Abuja in 1991. Other very effective

cultivars were TGX-1660-18F and TGX-1674-8F from Zaria and Mokwa respectively. Screenhouse experiments indicated that growing cotton (Abuja Local) or cowpea (Tvx 3236) in *S. hermonthica* infested soil the previous season significantly reduced parasitism on maize planted the following season and increased yields. Unemerged attached, emerged, total attached *S. hermonthica* and root dry weight were positively correlated with symptom severity on maize. All parameters were negatively correlated with grain weight, and all, except stem dry weight were negatively correlated with harvest index. Unemerged attached, emerged and total attached parasites on each maize plant decreased with increasing length of cotton or cowpea growth the previous season. No significant advantage was obtained by addition of fresh cotton or cowpea mulch.

Pot experiments with crop residues indicated that at all incubation periods of cowpea and soybean residues increasing weight of residue delayed *S. hermonthica* emergence, reduced total number of parasites attached per maize plant and increased plant height and total dry matter yield of maize. At all residue levels, incubation period of the parasite seeds and the residues for at least 7 days was required to significantly reduce parasitism of *S. hermonthica* on maize. Field experiments showed that all rotations significantly reduced the number of attached parasites on maize plants and increased grain yields the following season. Addition of residues from Tvx 3236 reduced parasitism of the parasite on maize and increased yields.

GENERAL INTRODUCTION

Maize (*Zea mays* (L.) is the most important staple food crop in Kenya and is grown in all agricultural zones. Sorghum (*Sorghum vulgare* (L.) and millet (*Eleusine corocana* (L.) are grown in medium and low agricultural zones. Maize production covers about 40 percent of the total area under crop production in Kenya. Small scale farmers are the major producers in terms of the area cropped, production and labor input. While small-scale farmers grow maize mostly for home consumption large scale producers account for the major share of the officially marketed maize.

Although no detailed survey of *Striga* species distribution has been made in Kenya, *Striga hermonthica* (Del.) Benth is predominant in the Lake Victoria basin. It occurs in all districts of Nyanza Province. In Western Province, *S. hermonthica* is found mostly in Busia District, the southern part of Kakamega District, and the southwestern part of Bungoma District. *S. hermonthica* causes damage in maize, sorghum, millet and sugarcane (*Saccharum officinarum* (L.) (Michieka and Ariga, 1989).

Infestation of *Striga asiatica* (L.) Kuntze was reported in the Coast Province where it causes serious damage to upland rice at Matuga Agricultural Research Station (Kiriro, 1991). *Striga* problem in Kenya is largely associated with human population growth, which currently stands at 3.0 percent per annum. Initially farmers used

traditional farming systems which involved prolonged fallow, rotations, and intercropping. These practices kept *Striga* species infestations to tolerable levels. As population pressure and demand for food production increased, land use intensified. With greater use of monocropping and little or no fallow, populations of *Striga* species have gradually increased and become threats to cereal production in Kenya. Farmers have also shifted from growing cereals like millet and sorghum, which produced relatively better yields under the parasite infestation, to maize. Maize, although high yielding, did not evolve under *Striga* pressure and therefore may produce no crop under heavy infestations by the parasite. The problem of *Striga* species is more widespread and serious in areas where both fertility and rainfall are low.

In Kenya, crop losses resulting from *Striga* parasitism may range from 70 percent to total crop failure, depending on the severity of infestation (Mumera, 1985). In *Striga* endemic areas of Kenya, the parasite is mainly controlled by hand hoeing. However, the parasite emerges four weeks after crop emergence and therefore escapes the first weeding, which is done two weeks after crop emergence. Except in large scale commercial maize farming, where farmers weed twice, small scale subsistence farmers only weed once and therefore *Striga* mature and seed thereby increasing seed bank in the soil the following season.

The existence of ecotypes of *Striga hermonthica* and host specifity

of these ecotypes require the use of complementary control methods. Host-parasite relationships should be understood fully to appreciate the effect of multiple cropping as well as the impact of crop rotations that involve the use of trap crops, which stimulate *S. hermonthica* seeds to germinate but are not parasitised. The introduction of early maturing varieties could ensure crop harvest before *S. hermonthica* reproduces and thus, provide an opportunity to destroy the parasite before land reverts to fallow. Resistant cereal varieties in rotation with some trap crops such as cotton, cowpeas and soybeans, previously selected for high *Striga* seed germination stimulation should be the most long-term approach. Most of these trap crops are being grown by farmers in *S. hermonthica* infested areas of Kenya. However, there still remains the establishment of the best method to use these trap crops to deplete *Striga* seed reservoir in the infested soils.

The answer to a successful cereal production in *Striga hermonthica* endemic areas in Kenya lies in good agronomic practices. Farmers with ample resources can do proper farm management but the problem lies with small scale farmers, who are the majority, and have limited resources. In order to solve *Striga* problem in small scale agriculture, consideration should be given to the development of appropriate technology and its application. In all previous experiments with *Striga* seed germination stimulants, no attempts have been made to isolate and evaluate stimulants from different plant parts or from different aged plants. The identification of

trap crop cultivars with high concentration of *S. hermonthica* germination stimulants would enable the farmer to include them in the rotation to control these parasites. More need to be known about the concentration of the stimulants in different plant parts such as root, stem and leaves at different ages of the trap crops. This would help researchers to determine the best method of managing trap crop residues to control *S. hermonthica* and improve the soil fertility status at the same time. These experiments were therefore designed to achieve the following objectives;

(1) To select, *in vitro*, cultivars of non-hosts of *S. hermonthica* for stimulation of germination of parasite seeds.

(2) To test method of isolation and determine the general types of stimulants by partitioning of aqueous and organic soluble fraction of root, stem and leave extracts of these crops at different plant ages.

(3) To determine effective germination distance and time of maximum *S. hermonthica* seed germination in relation to non-host growth and its residues.

(4) To determine the ability of each of these crops and cultivars at different stages of growth to deplete *S. hermonthica* seed bank in the soil when grown in rotation and their residues incorporated as mulch.

(5) To establish field effectiveness of trap crops and their mulches, previously selected in the laboratory, to reduce *Striga hermonthica* parasitism when grown in rotation with maize.

GENERAL LITERATURE REVIEW

Striga hermonthica (Del.) Benth. is an extremely damaging parasite in maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), millet (*Pennisetum glaucum* (L.) R. Br.), sugarcane (*Saccharum officinarum* L.) and upland rice (*Oryza sativa* (L.) in most regions of Africa (Saunders, 1933; Brown *et al*, 1951; Shaw *et al*, 1962 and Doggett, 1965), where witchweed causes yield losses ranging from 10 to 100 percent (Ramaiah, 1983). A single *Striga hermonthica* plant is able to produce up to 500,000 seeds which mature at different times (Andrews, 1947). The seeds do not all germinate at the same time, but continue to remain viable in the soil for up to 20 years (Fisher *et al*, 1990). The long viability in combination with high seed production result in severely infested fields.

Striga seed germination requirements

Parker (1984) reported that there is tendency for samples of *S. hermonthica* from West Africa to give relatively low germination in the first 6 months after collection. Valance, (1950) concluded that seeds of *S. hermonthica* like that of *S. asiatica* also require an after-ripening period. This requirement is an excellent adaptation of *S. asiatica* and *S. hermonthica* to the semi-arid tropics, which prevents the parasite seeds from germinating at the end of the rainy season in which they were produced (Doggett, 1984). Seeds of *Striga* species require a period of exposure to moisture condition

for 1 to 3 weeks, depending on temperature before they will germinate following exposure to a germination stimulant (Worsham, 1987). Reid and Parker (1979) confirmed that 23°C was a more favorable conditioning temperature for *S. hermonthica* samples from both West and East Africa and on both sorghum and millet hosts.

The effect of *Striga* species on crop yield

Parasitic angiosperms threaten the lives of over 100 million people in Africa, seriously in 17 countries and moderately in 25 (Mboob, 1986). Yield losses caused by weeds generally result from competition for limited resources such as light, water and soil nutrients. A conservative estimate of crop losses due to *Striga* species in Africa is 40 percent, representing an annual loss of cereals worth US$ 7 billion (Mboob, 1986). The losses, however, vary with countries depending on the ecological zones which they contain. In the Gambia, in a two year study, crop losses due to *S. hermonthica* was found to range from 20 to 35 percent (Carson, 1986). In Nigeria, losses of 10 to 91 percent with an average loss of 35 percent, in sorghum and maize yields have been attributed to *S. hermonthica* (Parkinson, 1985).

In Cameroon, 15 to 20 percent of overall production was affected by *Striga* species and the losses in certain cases were as high as 50 to 90 percent (Lagoke *et al*, 1991). Preliminary surveys at the farmer level in Mali showed that crop losses due to *Striga* species

ranged from 25 to 100 percent (Konate, 1986). In East Africa, Doggett (1975) estimated a 20-95 percent total yield loss for sorghum and millet. Experiments conducted in Sudan indicate that *Striga* infestations can cause almost complete crop failure while soil fertility is still adequate (Leroy, *et al*, 1977). The authors also noted that upland rice growing in areas of southern India has been stopped because of 80 to 90 percent crop losses from *Striga* species.

Information on the effects of *Striga* spp. on crop growth is still lacking (Ransom *et al*, 1990). Unlike other weeds, *Striga* attaches to the vascular system of the host plant and diverts carbohydrates produced by the host. It has been estimated that about 20 percent of the yield reduction in the host can be attributed to a loss of fixed carbon diverted to the attached *Striga* species. The symptoms of *Striga* parasitism on the host plant suggest a phytotoxic effect (Graves *et al*, 1989). The authors noted that in cereals such as maize and sorghum *Striga hermonthica* causes stunting, drought like leaf wilting, chlorotic lesions and leaf rolling even under high moisture condition. *S. hermonthica* has been noted to reduce leaf, stem and root growth of infected susceptible maize plant which also showed completely moribund shoots, with no green leaves some 76 days after planting (Stewart *et al*, 1991).

Stewart and Press (1990), have summarized the growth inhibiting effects of *Striga* species on its host, and speculated that a

''toxin'' produced by the parasite is responsible for the symptoms. Butler *et al* (1991) found that extract of *S. hermonthica* shoots are toxic to sorghum cells growing *in vitro*. The authors are now using the *S. hermonthica* extract as a screening technique to eliminate cultured sorghum cells which are susceptible to the extract, recovering only those variants which survive the treatment. These cells would be regenerated later into plants to be tested in the field for resistance.

Control measures for *Striga* species

Although various methods have been tried to control *Striga* species, Doggett, (1984) noted that it may not be possible to eliminate *Striga* species but populations can be reduced so that they cannot build up sufficiently to cause economic damage. Three control aspects to be considered include;

(1) Avoid spread of *Striga* species seeds to uninfested areas.
(2) Cleaning up *Striga* species infested lands and,
(3) Controlling the parasite in the growing crop.

Methods of *Striga* Prevention

The Spread of *Striga* species is difficult to control because of the plant's prolific ability of producing between 50,000 to 500,000 seeds/plant (Doggett, 1984). The seeds are minute (0.25mm long) and

one of the mechanism of dispersal was thought to be wind (Pieterse and Pesch, 1983). This assumption implies annual influxes of wind blown *Striga* seeds from infested areas to farmers' fields and suggests that methods to reduce yield loss should receive greater attention than in-field eradication. To clarify the situation, the relative importance of wind, cattle (through seed ingestion and deposition), and man (through contamination of planting materials) in seed dispersal was examined and wind was found to be relatively ineffective in long distance seed dispersal and is probably not responsible for influxes of *Striga* seed into farmers' fields. Only about 8 percent of *Striga* species seeds ingested by cattle remained viable, and these were deposited at a distance equal to or less than 0.5 Km, which is not far from the site of ingestion (Berner *et al*, 1994). This indicates that cattle are also relatively ineffective in long-distance *Striga* ssp. seed dispersal. However, the finding that up to 80 percent of *S. hermonthica* seeds may pass through the tracts of sheep without loss of viability (Bebawi and Elhag, 1983), makes it imperative that use of animals for grazing should be carefully regulated to minimize the spread of this weed.

Berner *et al*, (1994) also found that the average amount of *Striga* seed contamination of cowpea, maize, millet and sorghum seeds sold in local Nigerian markets was 29.8 to 63.4 *Striga* seeds per sample. This implies that the use of *Striga* free planting material is thus the first step in control and can be achieved by purchasing seeds from reputable seed companies, planting seeds harvested only from

Striga free fields, and drying and threshing harvested seeds only in *Striga* free fields. The spread of *Striga* spp. seeds by agricultural machinery and storm water have also been suggested (Saunders, 1933; Hosmani, 1978; Parker and Wilson, 1986).

In *Striga* endemic areas of Kenya, this parasite is often the only green vegetation seen in the field after harvest, which marks the beginning of dry season. Livestock survival depends on this parasite at this time of the year. Preventive measures should be taken by educating farmers on sanitary measures and information should be availed on seed production and spread of *Striga* species. Information on post-harvest management practices that prevent *Striga* plant from going to seed should be highlighted

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Controlling the parasite by hand pulling

Striga species emerge later than 4 weeks after sowing and upto 12 weeks where the rains are prolonged, and therefore escape the main weeding operation (Parker and Wilson, 1986). Uprooting *Striga* species by hand every 10 days to 2 weeks has been recommended but this is burdensome and not possible where infestation is high (Doggett, 1975; Parker and Wilson, 1986). When *Striga* plants are uprooted at early stages the weak stem easily breaks leaving roots and part of the stem in the soil. This results in regeneration from the laterals below (Akobundu, 1991). If the emerged *S. hermonthica* plants are removed, then some of the subterranean *Striga* appear in

their place as a result of less competition for nutrients (Last, 1960; Ishag, 1968 and Ogborn, 1972).

In Kenya, Watt (1936), reported that handpulling of *Striga* spp. was a compulsory farm practice which Kenyan farmers had to implement. Ramaiah and Parker (1982) also pointed out that *Striga* could continue to survive, mature, and set seed for several weeks after the death of the host shoot. The crop stubble should be uprooted after harvesting to prevent further growth of *Striga* species. With this method, the damage to the crop in the field is already done since the farmer has to wait till the *Striga* plant has emerged. There is the danger of spreading seeds when handpulling is done after the *Striga* plant has set seeds.

Effects of soil fertility on *Striga* species

Association of *Striga* species with condition of low soil fertility is not fully understood. Application of high rates of nitrogen fertilizer is generally beneficial in at least delaying *Striga* emergence and obtaining stronger crop growth (Bebawi, 1981; Doggett, 1984 and Mumera, 1983). On very infertile and heavily infested soils, nitrogen can increase the amount of the parasite emergence (Williams, 1961 and Last, 1960). In Kenya, application of nitrogen fertilizer at half the recommended rate of 39 Kg N/ha, in maize, reduced emerged *S. hermonthica* population by 64 percent

Solomon (1952) working with *Striga lutea* (Lour.) and *Striga densiflora* (Benth.) showed that if nitrogen level is low in the soil, the grain yield of sorghum is low and parasite attack is more severe. He measured the osmotic pressure of the plants under different fertility levels and found that the pressure in the parasite tissues was always much greater than that in the host tissues at a lower concentration of nutrients. At a higher concentration of nutrients, the pressure level in sorghum and the parasites tended to be the same. The higher osmotic pressure in the parasite compared to the host, at a low concentration of nitrogen in the soil, would favor the flow of water and nutrients from the host to the parasite.

Potassium, phosphorous, sodium chloride and magnesium sulphate appear to have little effect on *Striga senegalensis* parasitism on sorghum (Williams, 1961), but lime (Joglekar *et al*, 1959) and calcium nitrate (Williams, 1961) are said to be beneficial. Most of small scale farmers in developing countries who produce cereals for home consumption cannot afford the price of fertilizers. Moreover the availability and distribution of these fertilizers is not guaranteed. Rainfall unreliability compounds the problem of application of nitrogen to control *Striga* species.

Effects of time of sowing and irrigation on *Striga* species

In rainfed areas of East Sudan, late sowing (August, 31 onwards) of

sorghum is preferred to early sowing (June 9 to August 17) because the intensity of *S. hermonthica* infestation is reported to be much less with late than with early sown sorghum (Bebawi, 1984). The author noted that late sowing occur when the rains are well advanced and the soil temperatures have become relatively cool, whereas early planting dates occur when the rains are intermittent and soil temperatures are warm. He explained that the warm temperatures in the early season may be conducive to greater infestation of *S. hermonthica* compared to the cool temperatures of the late season.

Light irrigation of the sorghum during the normal sowing period was reported to increase *S. hermonthica* attack, whereas heavy irrigation decreased it (Andrews, 1945). Vallance, (1950) suggested that *S. hermonthica* seed would go into a state of wet dormancy when subjected to long periods of wetting. Water is not generally available for irrigation in *S. hermonthica* infested areas of Kenya. Where it is, the extra investment required to install irrigation equipment may not be afforded by small scale farmer.

Use of herbicides to control *Striga* species

The use of herbicides to control *Striga* spp. is a labor saving innovation. The ideal herbicide for any *Striga* spp. control is one that will protect a crop from attack by the parasite. This could be achieved if the herbicide would prevent germinated *Striga* spp.

seeds from attaching to the host, or if the attached seedlings are killed selectively following uptake of the herbicide from the host (Parker and Wilson, 1986, Akobundu, 1991 and Berner *et al*, 1994). Among the most promising pre-emergence herbicides to control *Striga* spp. include trifluralin, benefin, fluchloralin, pendimethalin (Anon, 1983; Ross and Lembi, 1985), oxyfluorfen , formesafen, dichlorobenil and DCPA (Eplee and Norris, 1987). Post emergence herbicides such as the salt formulation of 2,4-D (Yaduraju and Hosmani, 1979), ametryne, linuron, oxyfluorfen, formesafen, lactofen at low rate and gramoxone (Eplee and Langston, 1970).

Other herbicides tried to control *Striga* species include 2,4-DB; paraquat, atrazine, bromoxynil, MCPA; 2,3,6-TBA; 20% urea solution, fenac, basamid, metolachlor and dicamba (Joglekar *et al*, 1959; Ogborn, 1970. Bebawi and Farah, 1981; Mumera, 1983; Ramaiah, 1983; Eplee, 1984; Parker and Wilson, 1986). The herbicide imazaquin was tested for efficacy in *Striga gesnerioides* and *Alectra vogelli* control when applied as cowpea (*Vigna unguiculata* L.) seed treatment (Berner *et al*, 1994). By prolonging seed soak times at an imazaquin concentration of 1.8 mg/ml, good parasite control was obtained. Previous work with fertilizer herbicide combinations re-ported that nitrogen plus 2,4-D increased both grain and straw yield of sorghum (Last, 1960) but the use of 2,4-D combined with fertilizer seems to be of limited application, because of sensitivity of other crops such as cotton (Eplee and Langston, 1971). Work done by Bebawi and Farah, (1981) suggest that a

combination of a nitrogenous nutrient (nitrophoska) and herbicide (atrazine) are more effective in depressing the severity of *S. hermonthica* attack, and in stimulating the yields of sorghum, than sole treatments of either nitrogen or herbicide.

In Kenya the adoption of herbicides to control *Striga* and other weed species will depend on the availability and price. Herbicides are imported in Kenya from developed countries and this requires foreign exchange. As a result, price increases in herbicides have made their use for weed control unattractive. Some of the cropping systems may not be compatible with the herbicides recommended for *S. hermonthica* control and the spectrum of weeds to be controlled along with *S. hermonthica* need to be established. Herbicide safety during handling and rate and method of application should be taught to the farmers.

Effects of land preparation and sowing on *Striga* species

Saunders,(1933) has shown that wide spacing of maize plants has a considerable advantage in the management of *Striga asiatica* in heavily infested soils in South Africa. Increasing the space between sorghum plants in heavily infested soils in the Sudan resulted in large decreases in infestation of *S. hermonthica* in unmanured crops and small decreases in manured crops (Last, 1960). It is assumed that the stimulant excreted by roots of widely spaced plants may not be available in sufficient amounts or concentration

to cause germination of many *Striga* species seeds. In addition, host intraspecific competition for nutrients and moisture may be less under a wider spacing and host plants may be better able to withstand *Striga* parasitism (Bebawi, 1987). *S. hermonthica* incidence on sorghum has been observed to be less when sorghum was planted on ridged than on flat seed beds (Bebawi and Farah, 1981).

Tillage operations are not effective as means of controlling *Striga* species, which normally appear above ground in the later stages of crop growth, after causing considerable damage below the ground (Bebawi, 1987). Shallow ploughing of less than 15 cm was disastrous under *S. asiatica* infested conditions and ploughing deeper than 18 or 20 cm did not seem to offer any advantage. Saunders, (1933) and Doggett (1953), observed that *S. hermothica* did less damage on unploughed plots. However, Narasimhamurthy and Shivaramakrishnaiah (1963) showed that deep ploughing the land before planting decreased the incidence of attack by burying *Striga* species seed deep into the soil. Ali el Awad Mazlum (1983), reported that *S. hermonthica* seeds buried 50 cm deep in soil for three months lost their viability. The majority of small scale farmers in *S. hermonthica* infested areas of Kenya prepare their land by either hand hoeing or using ox-drawn ploughs. The soils in these areas are also hard when dry meaning that the ploughing depth is often less than 15 cm. Therefore, the use of deep ploughing to control the parasite is not feasible.

A combination of tied ridging (where sunken portion is surrounded by ridges on four sides) in combination with mulching has been used to control *S. hermonthica* (ICRISAT, 1982). The report indicated that use of tied ridges in sorghum and millet in Upper Volta resulted in almost doubling of crop yields without reducing *Striga* plant growth significantly. This indicates that tied ridges in West Africa provide better conditions for crop growth as well as for *Striga* because sufficiently wet conditions are not created to reduce the parasite emergence (Ramaiah, 1983). Mulch is not available in *S. hermonthica* infested areas of areas of Kenya, as crop residues are used to feed livestock after harvesting, which coincides with the onset of dry season.

Soil solarization which uses natural sunlight to disinfest the soil has been used in the control of *S. hermonthica* (Sauerborn *et al*, 1991). In this method, a clear polyethylene plastic sheet is placed over moist, well tilled soil. Soil is heated by incoming radiation (the greenhouse effect) while the plastic sheet retains soil moisture and reduces heat loss through evaporation (Braun *et al*, 1987). The authors noted that solarization should be practiced during periods of high solar radiation to be effective over a short period. A higher temperature of at least 40°C is required to reduce *S. hermonthica* seed viability (Sauerborn, *et al*, 1991). In their experiment it was noted that at 50 and 70 Klx, where the mean daily maximum temperature at 1 cm depth reached 47 and 55°C respectively, the viability of seed was drastically reduced after 1 day compared

with the 30 Klx treatment with a mean daily maximum temperature of 42°C. Beside the control of parasite seeds, soil solarization is also showing promise as a method of controlling soil-borne diseases, nematodes, and weeds (Pullman *et al*, 1981; Sauerborn and Saxena, 1987 and Jacobson *et al*, 1980).

Biological Control of *Striga* species

Biological control agents for *Striga* were recently reviewed by Bashir (1987). Available literature on this subject indicate that both macrobial and microbial methods of biological control look promising from preventing *Striga* plants from going to seed. While a lot is still to be learned about ecological requirement, quantitative damage, and host specifity, among the most promising control agents are: gall-forming insects (*Smicronyx* spp.) and the borers (Lepidopterous and weevil spp.). Much less is known about microbial control of *Striga* species. Some fungi (*Cercospora* and *Phoma* spp.) have shown promise as potential microbial control agents for *Striga hermonthica* (Zummo, 1977). One of the attractions of biological control is the safety to the environment. The development of mycoherbicides combined with insect control of *Striga* spp. would be the most appropriate (Akobundu, 1991).

Striga hermonthica (Del.) Benth., which is parasitic to cereals in Kenya, can be controlled by a local butterfly, *Junonia orithya L.* (Olela, 1993). He reported that the larvae eat up all the aerial

parts of *S. hermonthica* plant. Defoliation of *S. hermonthica* by *J. orithya* was also reported in Tanzania and Uganda (Greathead and Milner, 1971), Sudan (Bashir and Musselman, 1984), and in Upper Volta (Parker, 1987). Agarwal and Maquvi (1953) studied the species in their attempt to determine its suitability as a biological control of *Striga euphrasioides*. They established that the species is polyphagous and that besides *Striga*, it fed on *Ipomoea batatus*, which is widely grown in *S. hermonthica* regions of Kenya.

Breeding for resistance/tolerance to *S. hermonthica*

Striga resistance in maize can be defined as the capacity of maize to produce a satisfactory grain yield in the presence of many *Striga* seeds in the soil, whilst at the same time supporting fewer attached *Striga* plants than do susceptible maize (Doggett, 1984). Tolerant maize varieties will produce satisfactory yield amid heavy infestation of *Striga* spp. seeds in the soil and supporting as many attached *Striga* plants as susceptible maize variety (Kim, 1991).

Modern breeding methods and the availability of world germplasm collections have greatly improved the situation because better resistant types are becoming available (Doggett, 1984).
Use of *Striga* resistant and early maturing crop varieties are economical control practices. Early maturing varieties have the advantage of realizing maturity, thus producing grain, before *Striga* species reach their reproductive phases (Bebawi, 1987). This

may contribute to depletion of the reservoir of *Striga* seeds in the soil. New cultivars of resistant and early maturing crops would offer the farmer the opportunity to increase production at infested sites without changing the method of production.

Striga hermonthica (Del.) Benth., an obligate outcrosser, exhibits considerable intrapopulation variation in contrast to *Striga asiatica* (L.) O. Kuntze and *Striga gesnerioides* (Willd.) Vatke which are strongly inbreeders and are relatively uniform within a given population (Musselman *et al*, 1991). There is clear evidence of the presence of at least two strains, one specific to millet and the other to sorghum (Wilson-Jones, 1955; Parker and Reid, 1979; Bebawi, 1981; and Ramaiah, 1984). The crop specifity is reported to be based on the type of germination stimulants compounds present in the host crop root exudates (Parker and Reid, 1979). Where one cereal is affected by *S. hermonthica*, it is possible that another cereal would be grown thus, meeting the need to have sufficient land under cereals, which form large part of carbohydrate source to a large population in Kenya.

Saunders (1933), was the first to study the causes of resistance to some of the South African sorghum varieties resistant to *S. asiatica*. He noted that there were mechanical and physiological barriers in the roots of resistant varieties. In some of these varieties, extra lignification in the pericycle cells at the contact point of the haustorium was observed which prevented *S.*

asiatica haustoria from entering the stele (Maiti *et al*, 1984).

Resistance based on low *Striga* germination stimulant production has also been reported (Parker *et al*, 1977). In Nyanza Province of Kenya, two early high yielding sorghum cultivars, Kano and Dobbs, though not resistant to *S. hermonthica*, were reported to be producing a good crop before the parasite had taken a firm hold (Watt, 1936). Maize is a New World crop and has not been subjected to *Striga* species during its evolutionary course (Ramaiah, 1987). The author suggested that it is unlikely that any resistance mechanisms have evolved. *Striga* virulence trials in several African countries revealed that maize cultivars were susceptible to both sorghum and millet specific strains of *S. hermonthica* (Ramaiah, 1987). Selection within self-fertilized lines of maize has led to slightly increased resistance, but on the whole the results were not encouraging (Saunders, 1933).

In recent years, maize hybrids have been screened for resistance/tolerance to *S. asiatica* (Eplee, 1981) and *S. hermonthica* (Kim, 1991). In 1983, genes for *S. hermonthica* tolerance were discovered when significant genetic variation was observed among IITA maize hybrids and inbreds (IITA, 1984; Kim *et al*, 1985). Most of the tolerant varieties, the best being 8322-13, were crosses between tropical and U.S Corn Belt materials (IITA, 1984; Kim *et al*, 1985). A few lines previously reported as tolerant showed segregation for resistance to *S. hermonthica* (Kim, 1991). It

has been noted that a tolerant crop variety has a disadvantage in that it encourages the build up of *Striga* seeds (Doggett, 1984; Kim, 1991). Maize tolerant varieties may be more stable than a variety with the single gene type resistance, which could be overcome by mutant biotypes of *S. hermonthica* (Kim, 1991).

Synthetic *Striga* germination inhibitors and stimulants

The most effective soil fumigant to deplete *Striga* species seeds from the soil is methyl bromide, which requires skilled labor for its application (Eplee and Langston, 1971). Metham sodium, a liquid fumigant and dazomet, a granular material have also been used (Eplee and Norris, 1987). Some purines, coumarines and ethylene had been the first chemicals identified in laboratory screenings to induce germination of *S. asiatica* (Worsham *et al*, 1959 and 1962; Egley and Dale, 1970). A single ethylene gas treatment can rid the soil of up to 90 percent of the viable preconditioned *Striga* species. seeds (Eplee, 1975). The author reported that mechanical equipment for ethylene soil injection developed in USA works well. The back-pack equipment, however, would take a very long time to cover one hectare (Doggett, 1984). If the price is affordable the use this technology is possible on small subsistence farms in Kenya.

A stimulatory chemical was isolated from root exudate of cotton (*Gossypium hirsutum* L.) by Cook *et al*, (1966), and its structure

was elucidated by X-ray crystallographic analysis (Cook *et al*, 1972; Goggon *et al.*, 1973). The compound which was given the name strigol, is a very potent germination stimulant for *S. asiatica*, other *Striga* species and similar parasitic plants in the genus *Orobanche* (Cook *et al*, 1966 and 1972; Johnson *et al.*, 1976 and Hsiao *et al*, 1981).

Striga spp. seed germination stimulants have been synthesized and tested for activity (Cook *et al*, 1972 and Pepperman *et al*, 1982). The name for these compounds is 'strigol'. The authors noted that among the compounds, GR 7 and GR 24 are the most stable and active and that seasonal effects were found in the germination percentages of *S. hermonthica* seeds with GR 24. The most interesting combination tested in USA was the use of GR 24 in combination with either the herbicides paraquat or oxyfluorfen, which was as effective as soil injection with ethylene when used on bare land (Norris and Eplee, 1981).

It has been reported that pre-sowing hardening of sorghum seeds with phenolic compounds such as caffeic, ferulic, and vanillic acids reduced *S. asiatica* seed germination under laboratory condition, but not under field condition (Bharathalakshimi and Jayachandra, 1980; Friesen and Korwar, 1990). Kinetin and several related cytokinins have been reported to induce *S. asiatica* germination and haustorial initiation (Worsham *et al*, 1959; Riopel and Baird, 1987). Thiodiazuron, a substituted urea, was reported to

have a high intrinsic cytokinin activity in several bioassays (Mok *et al*, 1982) and induced germination and haustorium initiation in *S. asiatica* and *S. hermonthica* (Babiker *et al*, 1991).

Catch and trap crops as natural *Striga* germination stimulants

Trap and catch cropping system are the foundation of an efficient cultural strategy of reducing *Striga* species seed in the soil (Bebawi, 1987). The author described catch crops as those plants that have the ability to germinate *Striga* species seed, but can also be parasitized. In catch cropping, both host and parasite are destroyed before the *Striga* species flower (Parker and Wilson, 1986 and Bebawi, 1987).

Catch crops tend to cause a higher percentage of *Striga* spp. seeds to germinate than trap crops, and it may be worthwhile to sow catch and trap crops in alternate but closely spaced rows and weed out the catch crop a month or so after sowing (Parker and Wilson, 1986). Sudan-grass (*Andropogon sorghum*) is a very effective catch crop and growing it for five weeks before sowing sorghum greatly reduced infestation of *S. hermonthica* in East Africa (Last, 1960). One of the merits of catch cropping is that it is equal to green manuring in its restorative effect on the soil. In addition, catch crops are normally used as fodder crops in the rotation. One of the major drawback of catch cropping is that if the soil is wet, the cultivation and destruction of both host and parasite becomes

physically impossible. In Kenya, catch cropping is unlikely to be practiced in the rainfed areas since the season is short in the *Striga asiatica* and *S. hermonthica* infested areas.

Trap crops (false hosts) are those plants which stimulate germination of *Striga* species seed without being parasitized and are consequently allowed to mature and produce a crop (Bebawi, 1987). Trap cropping is preferred by most farmers because of the yield obtained from them. However, *Striga* species seed longevity in the soil may hamper its adoption. Studies indicated that *Striga asiatica* seed may remain germinable in the soil for 14 years or more and normally, seed longevity increases with depths in the soil (Saunders, 1933 and Bebawi *et al*, 1984).

Some Trap crops for *Striga asiatica* include soybean (*Glycine max* L.) and field beans (*Phaseolus vulgaris* L.), (Robinson *et al*, 1966); sunflower (*Helianthus annus* L.), cowpeas (*Vigna unguiculata* L.), linseed (*Linum usatatissimum* L.), castor-bean (*Ricinus communis* L.), (Hattingh, 1956) and cotton (*Gossypium* spp.) (Narasimha *et al.* 1957). *Striga hermonthica* parasitizes a number of species but soybean, lucerne (*Medicago sativa* L.) and sun-hemp (*Crotalaria juncea* L.) and dolichos bean (*Dolichos lablab* L.) (Andrews, 1947) and cotton (Doggett, 1953) are suitable trap crops. Other trap crops documented include pigeon pea (*Cajanus cajan* L.), green gram (*Phaseolus aureus* L.), black gram (*Phaseolus mungo* L.) and sesamum *(Sesamun indicum L.)* (Andrews, 1947 and Hosmani, 1978).

The authors noted that further documentation of the efficacy of the trap crops in reducing soil population of *Striga* spp. is needed. Differences in varietal reaction should not be forgotten (Andrews, 1947). In Kenya, it was noted that growing of cassava crop for three years almost freed the land of *S. hermonthica* (Watt, 1936).

Several workers recommend the use of trap crops in the rotation to deplete the reservoir of witchweed seed in the soil (Andrews, 1947; Doggett, 1970; Ramaiah and Parker, 1982; and Wilson-Jones, 1953). Parkinson *et al*, (1988) showed that the *S. hermonthica* population in the plot under three years continuous cropping with soybean was significantly lower than the plot under continuous maize for a similar period of time. Bambara nut was found to be another suitable trap crop in the drier Sudan and Sahel savannas where neither cotton nor soybean can be grown as trap crops. In another experiment, to study the induction of *S. hermonthica* germination by root exudates, Bebawi and Michael, (1991) reported that hyacinth bean (*Dolichos lablab* L.) had the highest stimulation followed by cotton (*Gossypium barbadense* L.), guar [*Cyamopsis tetragonoloba* (L.) Taub.], sesame (*Sesamum indicum* L.) and sunflower (*Helianthus annus* L.).

In severely infested fields, one to several years may elapse before the *Striga* spp. population is reduced to non damaging levels. Where groundnut (*Arachis hypogaea* L.), field beans, sunhemp (*Crotalaria juncea* L.) and buckwheat (*Fagopyrum esculentum* L.) were grown

previous to corn (*Zea mays*) crop, Rose and Rochrie, 1941, showed that ''there was good reason for suspecting that these trap crops had actually destroyed much of the *Striga* seed by causing it to germinate '', following germination of these seeds in the absence of a host.

In the Sudan, Andrews (1945) from pot experiments, and later Wilson-Jones (1953) and Last (1961) from field experiments, have found that the sowing of groundnut, cowpea, and dolichos bean in a *S. hermonthica* infested soil did reduce a large proportion of the parasite seed. In India, in a pot experiment , Yaduraju and Hosmani (1979) reported that all trap crops including cowpeas, groundnuts, and linseed decreased the incidence of *S. asiatica* on the succeeding crop with cowpea and groundnut being the most effective.

A number of non-cultivated hosts of *Alectra vogelii* have been documented. In South Africa *A. vogelii* has been found growing on *Acanthospermum australe* (Loefl.) by Hattingh (1954). In Botswana, examination of roots of the widely occurring *Acanthospermum hispidum* during 1981/82 season showed that stands of this weed were also parasitized (Riches, 1988). However the last author noted that the parasite developed poorly on *A. hispidum* following attachment. *A. vogelii* was also found growing and flowering on *Vernonia poskeana*, *Indigofera spp.*, and *Tephrosia purpurea* (Riches, 1988). This indicates the role of weeds in the control of parasitic angiosperms. The induction of parasitic angiosperms to grow by

these weeds is a proof that they exude germination stimulants.

Isolation and testing of *Striga* seed germination stimulants

Previous work on recovery, isolation and identification of the natural germination stimulants, although without success, showed that even extremely small amounts of these substances induce maximum germination (Herb *et al*, 1987). Saunders (1933) demonstrated that leachates from pots in which sorghum plants were growing stimulated *Striga asiatica* germination, and it has been well established that roots of many host and non-host plants contain or exude factors that stimulate germination of a number of preconditioned *Striga* species seeds (Brown, 1946).

A large number of investigators have attempted to isolate and characterize and/or identify the stimulant(s) from many different host and non-host plants (Worsham *et al*, 1964, Khalaf *et al*, 1991 and Mallet, 1973). ''Sorgoleone'' is the first *S. asiatica* seed germination stimulant to be isolated and identified from the roots of sorghum, a natural host (Netzly and Butler, 1986; Chang *et al*, 1986 and Netzly *et al*, 1988). Capsaicin, the active ingredient of hot pepper, has a structure somewhat similar to sorgoleone and is active as a *Striga* germination stimulant, but Urushiol, which is structurally even more similar, is inactive (Riopel, 1991).

By means of paper partition chromatography and a suitable bio-

logical test, the number and variety of the *Orobanche minor* and *Striga hermonthica* germination stimulants produced by maize roots have been assessed (Sunderland, 1960). The author has shown that extracts of maize roots and aqueous solutions in which roots have been suspended (diffusate solutions) contain a complex of stimulating substances consisting of at least, one water soluble and a number of ether-soluble stimulants. The complex of ether-soluble stimulants, but not the water soluble stimulants, accumulate in a solution of glucose when it is exposed to fragments of maize roots.

In another experiment, Khalaf *et al*, (1991) found that flax germination stimulant(s) was soluble in highly organic solvents such as petroleum ether, hexane, benzene and ether, whereas, faba bean stimulant(s) was soluble in benzene and ether only. The presence of more than one natural *Orobanche minor* stimulant(s) in faba roots and flax seed diffusate was noted by the same authors. Certain legumes were found to stimulate germination of *Striga gesnerioides in vitro* and yet were not parasitized in the screenhouse experiments (Wild, 1948; Parker and Reid, 1979; Igbinnosa and Okonkwo, 1991). In host specificity, the production of active stimulants and the defence mechanism of the host plant play an important role. Igbinnosa and Okonkwo (1991) suggested that if a plant produces an active stimulant but does not have defence mechanisms, it is parasitized. The induction of suicidal germination by applying germination stimulants in very low dosages

in the absence of a host will eventually result in a more effective method of controlling *Striga* spp. with natural and environmental conserving method.

Cultivar differences exist among trap crops in their capacity to stimulate *S. hermonthica* seed germination therefore, it is necessary to do pre-selection in the laboratory. A quick, simple and cheap laboratory bioassay to screen trap crops should be established. This would reduce time and cost involved in field screening. The nature and distribution of germination stimulants in plant parts of trap crops should be well understood in order to exploit avenues for their future use, either as analog sprays or in crop rotation to reduce *Striga hermonthica* seed bank in the soil.

CHAPTER 1: EFFECTS OF NON-HOST EXTRACTS AND STIMULANTS ON GERMINATION OF *STRIGA HERMONTHICA* SEEDS

ABSTRACT

Striga hermonthica (Del.) Benth. is a hemiparasite angiosperm and its seeds are stimulated to germinate by compounds present in the root exudates from host and non-host plants as well as by synthetic germination stimulants. Laboratory results indicated that extracts of cowpea roots and shoots stimulated about 30 to 40% as much germination as 10^{-2} mgL^{-1} GR 24. No differences were observed among cowpea cultivars in germination percent of *S. hermonthica* seeds induced by the aqueous extracts. The population from Mokwa responded most to the extracts.

Increasing concentrations of both whole cowpea plant extracts and GR 24 resulted in increased *S. hermonthica* seed germination upto 6.3mg plant tissue mL^{-1} of water and 10^{-2} mgL^{-1} respectively, beyond which germination decreased. Higher germination was recorded when seed conditioning period was 11 days and incubation time of *S. hermonthica* seeds with GR 24 at 30°C was 48 hours in microtiter wells. Storing plant extracts at 7°C for 20 days did not reduce potency.

Results showed that cotton roots contained the most effective *S. hermonthica* seed germination stimulant among the plant materials

tested. The best conditioning period of the parasite seeds before bioassay with cotton extracts was 11 days. *S. hermonthica* seeds showed a higher response to aqueous extracts from cotton plant parts and their mixtures than *S. aspera* seeds. With both parasites, extract mixtures depressed germination of the seeds below the average induced by individual extracts in the mixture. Some extracts and mixtures were as effective as 10^{-2} mgL^{-1} of GR 24.

There was wide variation among plant extracts of cultivars of soybeans tested in stimulating germination of *S. hermonthica* seeds. TGX-1674-1F was as effective as 10^{-3} mgL^{-1} GR 24 in inducing germination of *S. hermonthica* seeds collected from sorghum in Zaria, Mokwa and Abuja in 1991. Other very effective cultivars were TGX-1660-18F and TGX-1674-8F for the parasite seeds collected from Zaria and Mokwa respectively.

1:1 INTRODUCTION

Many environmental factors affect the growth and development of *Striga*. Effects vary with the level or intensity of the factor and the growth and development stage of the parasite. Samples of *S. hermonthica* seeds from West Africa have been noted to give relatively low germination in the first 6 months after collection, an indication of an after-ripening requirement period (Parker, 1984 and Valance, 1950), and an ecological adaptation in the semi-arid tropics (Doggett, 1984). Seeds of *Striga* species require a period of exposure to moisture condition for 1 to 3 weeks and a temperature of 23°C before they will germinate following exposure to a germination stimulant from host and non-host plants (Andrews, 1947; Brown, 1965; Reid and Parker 1979; Sahai and Shivanna, 1982; and Worsham, 1987).

The physiological mechanisms involved in conditioning which make the seed responsive to germination stimulants are not well understood (Worsham, 1987). It has been proposed that conditioning may promote synthesis of germination stimulant, cause leaching of chemical inhibitors from the seed and increase the permeability of a structure within the seed, apparently the aleurone layer. It has been shown that a low water seed/ratio during conditioning of *S. asiatica* seeds resulted in higher germination percentages than a high seed/water ratio (Mangnus *et al*, 1992).

In nature, shoot development and further plant growth takes place only after the radicle penetrates the host root and establishes a conductive channel with it (Rogers and Nelson, 1962; Okonkwo, 1966; Okonkwo and Nwoke, 1978). Brown (1946) and Brown and Edwards (1946) proved that *S. asiatica* seeds produced a germination stimulant during pretreatment. Rogers and Nelson (1962) hypothesized that the stimulant produced by the seed is similar to that supplied by the host. The amount produced however, is insufficient as exogenous stimulant is required. In maize (*Zea mays* L.), the exudation of the germination stimulant(s) for *S. asiatica* and *S. hermonthica* is in the region of 3 to 6 mm behind the root apex (Worsham *et al*, 1964 and Sunderland, 1960). Little stimulant was found in the shoots of maize, and growing maize seedlings in the darkness increased exudation of the stimulant (Worsham, 1961). The germination stimulants for *Orobanche* species were not present in cells from older region of the host roots (Hameed *et al*, 1973).

Stimulants from different hosts seem to have synergistic effects (Sunderland, 1960). Separate leachates of maize and linseed roots induced germination of only 4 and 5 percent, respectively, of the *S. hermonthica* seeds tested. When the two extracts were mixed, they induced up to 51 percent germination. This was not merely a function of concentration, since increasing the concentration of maize extract alone had no such stimulatory effect. The synergistic effect is further complicated by the fact that there are inhibitory as well as stimulatory substances in the host root exudates

(Mallet, 1973). In the germination of *Orobanche minor* and *O. crenata* increasing the concentration of host root extract increases germination to an optimum level, beyond which germination is reduced (Brown *et al*, 1951; Mallet, 1973; and Whitney, 1979). Some of these researchers suggested that this was due to the presence of both stimulatory and inhibitory substances in the extracts; the response being independent on the ratio of the stimulant to the inhibitor.

Three non-host, cotton (*Gossypium* spp.), cowpea (*Vigna unguiculata* (L.) Walp.) and soybean (*Glycine max* (L.) are farmer acceptable and show promise in African farming systems. However, cultivars of these crops vary in their ability to germinate *S. hermonthica* seeds. Also, different plant parts may vary in their germination ability. To develop recommendations for effective *S. hermonthica* control with these crop rotations more needs to be known about effects of different cultivars at all stages of growth, plant parts, and relative concentrations of plant part exudates on *S. hermonthica* seed germination. This study was conducted to determine the effects of different sources of plant extracts and stimulants and varying conditions and concentrations on germination of *S. hermonthica* seed.

1:2 MATERIALS AND METHODS

Laboratory work was done at the headquarters of the International Institute of Tropical Agriculture, Ibadan (3.54 E, 7.30 N) and 213 m above sea level. Ibadan has a bimodal rainfall pattern with the main growing season between April and August and the short rainy season from August to October. There is a long dry season from November to April. The mean annual rainfall is 1278 mm and the mean annual temperature is 26.2°C.

1:2:1 Preparation of aqueous extracts and stimulants

Cotton (*Gossypium* spp.) and cowpea (*Vigna unguiculata* (L.) Walp. were grown in the screenhouse on ridges 75 cm apart and 25 cm within the ridge, for 120 and 90 days respectively. The crops were uprooted and the roots washed. Cowpea roots and shoots were combined and cotton roots, stems and leaves were tested separately. The samples were oven-dried at 60°C for 3 days and ground to pass through a 2 mm sieve. Samples of 5 g of each test material were mixed with 400 ml of distilled water for cowpea cultivars and cotton plant parts. In a separate experiment, extract concentrations were prepared by mixing 5 g samples of cowpea with 50, 100, 200, 400, 800, 1000, 1500, and 2000 ml of distilled water. Each sample was stirred and left to stand for 1 hour at room temperature. The samples were filtered through a funnel lined with Whatman No.1 filter paper and the volume of the filtrate recorded.

The extracts were stored at 7°C for 20 days and later tested on germination of *Striga hermonthica* seeds. A stock solution of 10^{-1} mgL^{-1} of GR 24 (3-((2,5-dihydro-4-methyl-5-oxo-2-furanyl 1) oxy) methylene)-3, 3a, 4, 8b-terahydro-2H-indeno (1,2-b) furan-2-one) was prepared and serially diluted to give concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} mgL^{-1}. These extracts were later tested on germination of *S. hermonthica* seeds conditioned for 4, 7, 14, 21, 30, and 42 days.

1:2:2 Conditioning *Striga hermonthica* seeds

Three *S. hermonthica* seed populations collected from sorghum plants in Zaria (11.07 N, 7.44 E), Mokwa (9.35 N, 5.11 E) and Abuja (9.12 N, 7.20 E) in Nigeria in 1991 were used in the experiment. Seeds were surface sterilized with 1 percent sodium hypochlorite solution. Three drops of ''Tween 80'' (Polyoxyethylene (20) sorbitan mono-oleate), a detergent, were added. This would facilitate submergence of tiny seeds (Okonkwo, 1987) and reduce contamination during preconditioning and stimulation process (Mangnus *et al*, 1992). After shaking, the solution was allowed to stand for 5 minutes. Floating seeds were decanted and discarded to eliminate immature or damaged seeds. The seeds were rinsed several times with sterile distilled water until the chlorine smell disappeared.

The seeds were washed in a funnel lined with Whatman No.1 filter

paper to dry. Two 9.0 cm filter papers were placed in each petri dish and moistened with sterile distilled water. Disks of glass microfiber filters (GF/A) of 8.0 mm diameter were arranged on top of the filter paper. Glass filter paper (Whatman GF/A) is important in reducing fungal contamination (Parker *et al*, 1977). *S. hermonthica* seeds were carefully sprinkled on the filter discs (25-50 seeds per disc). The petri dishes were incubated in the dark at 28°C for 10 days, before exposing the parasite seeds to germination stimulants or plant extracts.

1:2:3 *Striga hermonthica* seed germination bioassay

Two (Whatman No.1) filter papers were placed in a 9.0 cm sterile petri-dish. Each petri dish waş divided into three sections for the three *S. hermonthica* seed populations. The filter papers were moistened with 3 ml distilled water to provide partial air seal. Discs containing the parasite seeds were removed from petri dishes used for conditioning and dabbed on dry filter paper to remove excess moisture. Four discs each of *S. hermonthica* seed populations were arranged in each section of each petri dish. One germination stimulant or extract was tested in each petri dish by applying 0.13 ml of the stimulant or extract per disc, using a micro-pipette. A reference compound, strigol analog, GR 24 and distilled water were included in all test series as controls.

It has been noted that even with a standard bioassay test,

germination percent of *S. hermonthica* and *S. asiatica* obtained with GR 24 solutions vary from test to test (Mangnus *et al*, 1992). The author also noted that even local laboratory conditions and seasonal effects may interfere with the germination response of *S. hermonthica*. DMSO has been found to be less inhibitory than acetone (Pavlista *et al*, 1979). The petri dishes were sealed with paraffin film and incubated in the dark for at 30°C for 24 and 48 hours for comparison. In a separate trial stimulants were tested on discs or in sunken wells for comparision. Use of filter paper or not was tested in a separate experiment. Germination was recorded using a binocular microscope at X20 magnification. Relative germination percentages were calculated by expressing germination induced by the plant extracts as percent of that induced by GR 24, 10^{-2} mgL^{-1}.

1:3 RESULTS

1:3:1 Germination of *Striga hermonthica* seeds by GR 24

Results indicated that *S. hermonthica* seeds exposed to different concentrations of GR 24 after conditioning in the dark at 28°C for a number of days showed similar response to stimulant concentrations (figure 1). At all levels of conditioning, increasing the concentration of GR 24 up to 10^{-2} mgL^{-1} increased germination of *S. hermonthica* seeds up to this optimum after which germination decreased. Significantly higher germination was recorded after conditioning for 4 days compared to other treatments at low concentration levels. However, conditioning for 21 days gave the highest germination percent after exposing the seeds to a concentration of 10^{-2} mgL^{-1} of GR 24. Conditioning for 42 days gave significantly lower germination percent at all concentrations of GR 24.

In another experiment incubation time of *S. hermonthica* seeds, from different sources, with GR 24 at 5 X 10^{-3} and 10^{-2} mgL^{-1}, in sunken wells, were compared. Seeds from Zaria and Mokwa had significantly higher germination percent than those from Abuja (figure 2).
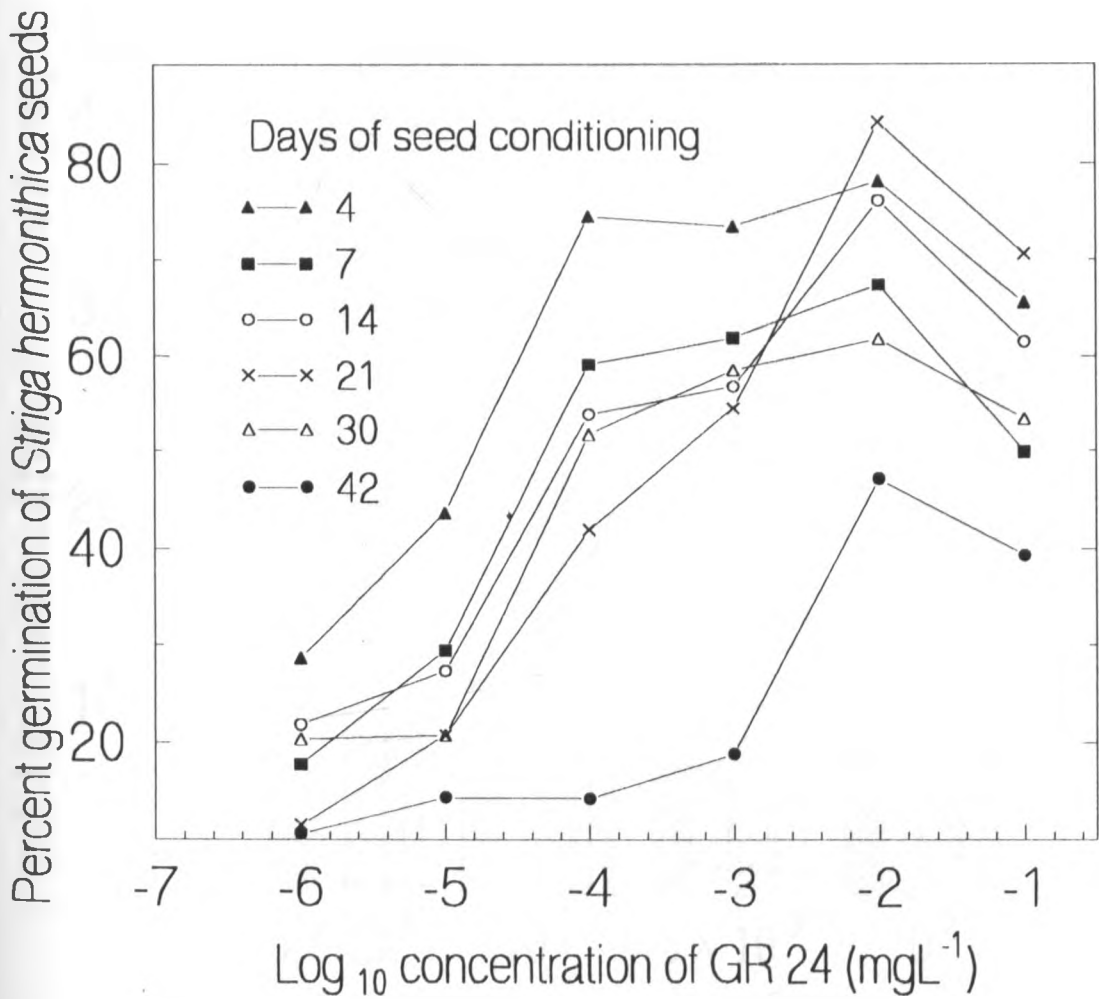
Fig. 1: Effects of concentration of strigol analog GR 24 on germination of
Striga hermonthica seeds conditioned in the dark on moist
glass fiber disks for 4, 7, 14, 21, 30, and 42 days at 28 ^{0} C.
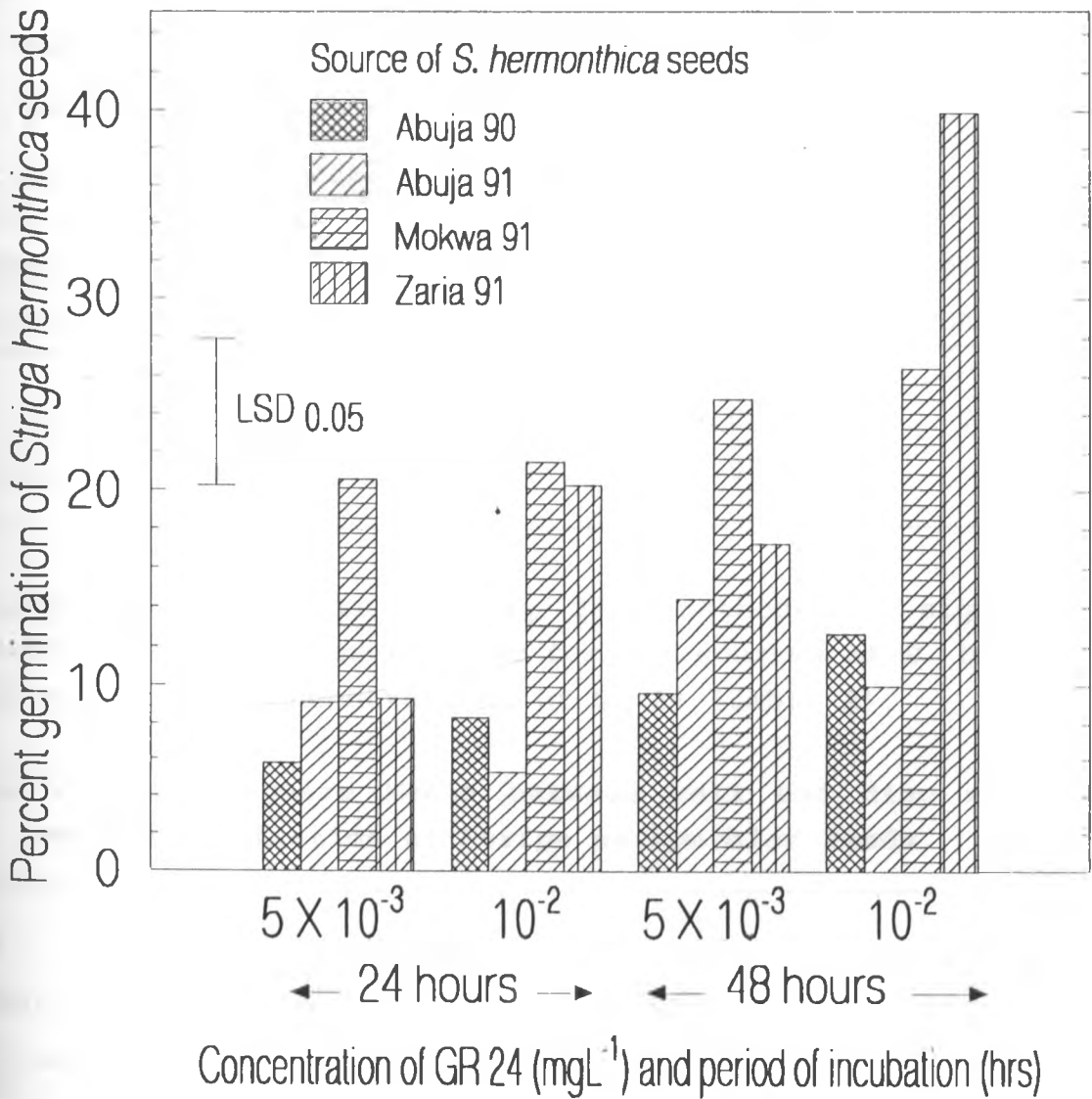
Fig. 2: Germination of *S. hermonthica* seeds in response to concentration of GR 24 (mgL^{-1}) and period (hrs) of incubation with the stimulant in wells.

striga seed germination significantly increased with increase in concentration of GR 24 from 5 X 10^{-3} to 10^{-2} mgL^{-1} and with increase in the period of incubation with the stimulants from 24 to 48 hours (table 1).

Table 1: Least Square Means showing response of *S. hermonthica* seeds to different incubation periods before recording germination percent induced by GR 24, 5 X 10^{-3} and 10^{-2} mg L^{-1} respectively in multi-wells.

INCUBATION PERIOD(HRS)	GERMINATION LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	Pr > |T| H0: LSMEAN1=LSMEAN2
24	14.5	1.8	0.0001	0.0013
48	21.3	1.8	0.0001	

Testing the stimulants using disc method was significantly superior than using sunken wells for all sources of *S. hermonthica* seeds and at two concentrations of GR 24 (table 2).

Table 2: Least Square Means between well and disc methods of testing germination of *Striga hermonthica* seeds.

TEST METHOD	GERMINATION LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	Pr > |T| H0: LSMEAN1=LSMEAN2
disk	63.5	2.1	0.0001 .	0.0001
well	39.8	2.1	0.0001	

significant differences (P=0.05) in the capacity of induction of *S. hermonthica* were detected among different sources of the parasite seeds, concentration of GR 24 and method used to test the stimulants (figure 3). Parasite seeds collected in 1991 from Zaria had highest germination response to 5 X 10^{-3} and 10^{-2} mgL^{-1} of GR 24 at both incubation methods followed by seeds collected in Mokwa and Abuja the same year. Increasing the concentration of GR 24 from 5 X 10^{-3} mgL^{-1} to 10^{-2} mgL^{-1} significantly increased germination of all the seeds at both incubation methods.

1:3:2 Germination of *S. hermonthica* seeds by cowpea extracts

Aqueous extracts of cowpea roots and shoots stimulated about 30 to 40 percent as much germination as GR 24 at 10^{-2} mgL^{-1} (figure 4). Germination induced by GR 24 at both concentrations was significantly higher than that of aqueous extracts from the three cowpea cultivars. Percent germination of *S. hermonthica* seeds induced by GR 24 at 10^{-2} mgL^{-1} was significantly higher than that induced by the same stimulant at 5 X 10^{-3} mgL^{-1}. Source of the parasite seeds determined the response to the aqueous extracts and GR 24. The order was Zaria > Mokwa > Abuja. No differences were observed among cowpea cultivars in germination percent of *S. hermonthica* seeds induced by the aqueous extracts.
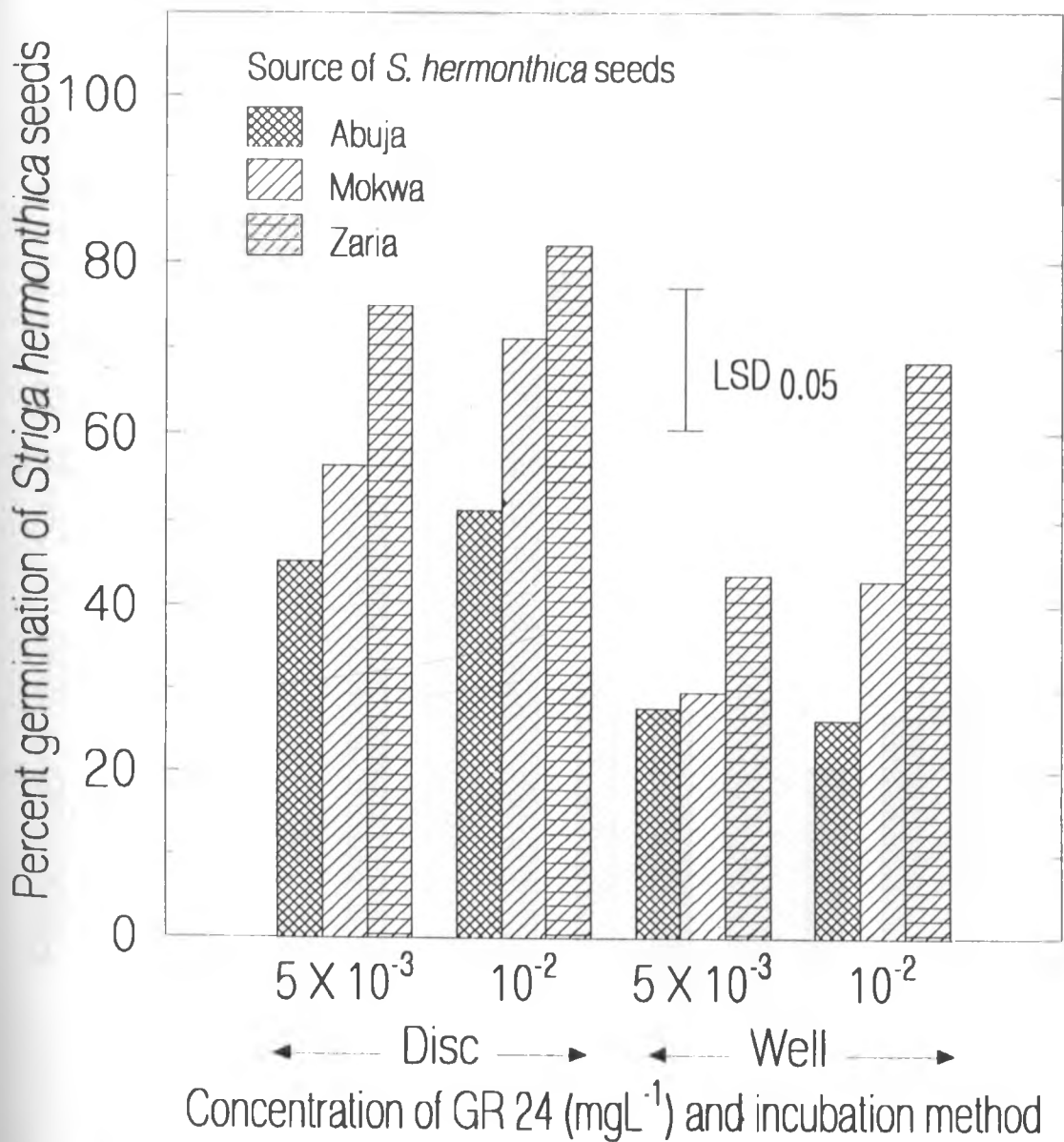
Fig. 3: Percent germination of *Striga hermonthica* seeds in response to concentration of GR 24 and incubation method
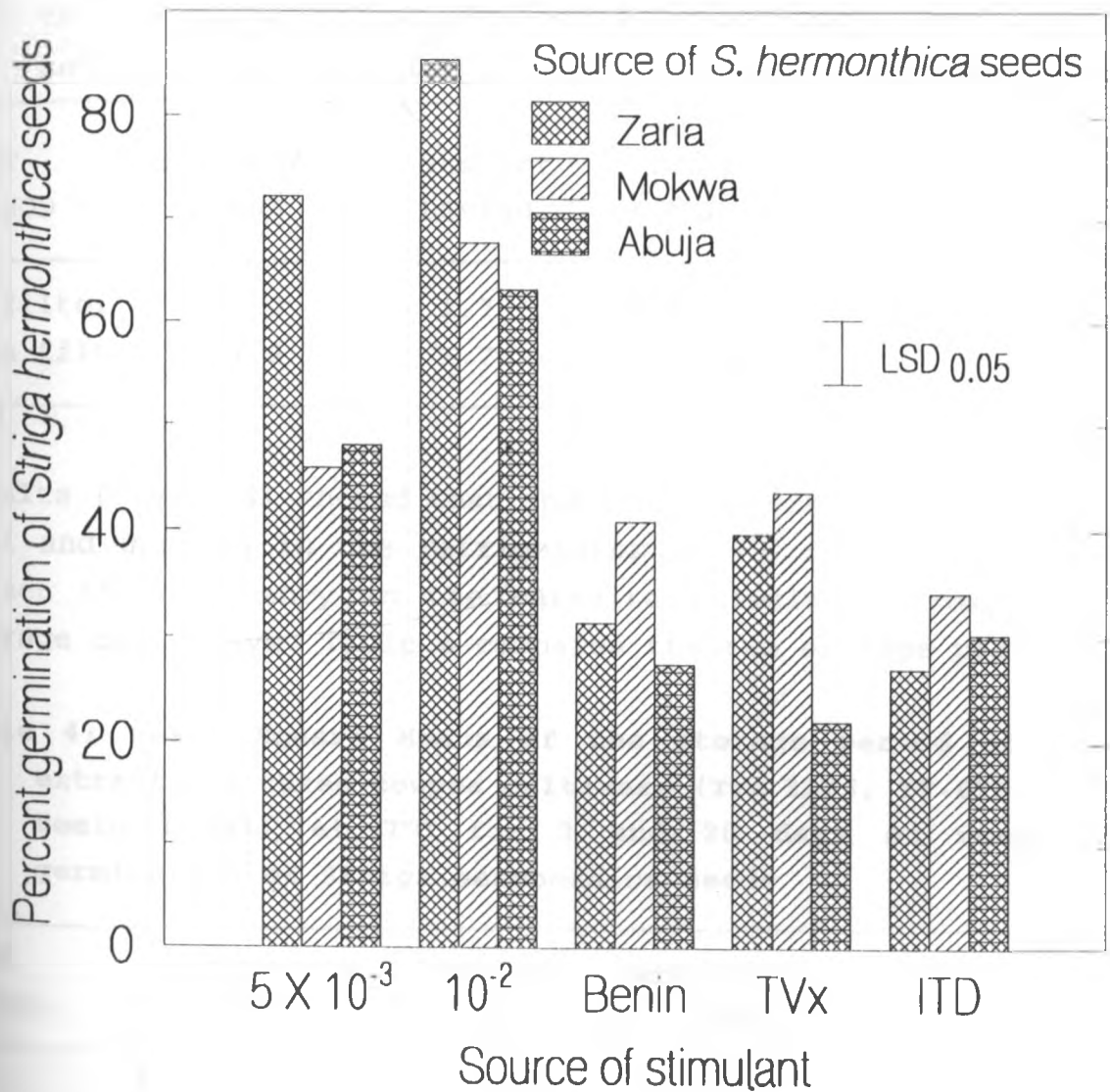
Fig. 4: Germination of *S. hermonthica* seeds in response to aqueous extracts from dried roots and shoots of three cowpea cultivars (Benin Local, Tvx-3236 IT-87D-1951) and in response to strigol analog GR 24 (mgL^{-1}).

Results (figure 5) indicate that placing two filter papers in the petri-dish, then placing 8 mm discs containing the parasite seeds before applying GR 24 or cowpea extract did not significantly increase germination of the parasite seeds over the same treatments without the filter papers (table 3).

Table 3: **Least Square Means of two testing methods of inducing germination of *S. hermonthica* seeds by aqueous extracts of three cowpea cultivars (Tvx 3236, IT-87D-1951 and Benin Local) and GR 24 (5 X 10^{-3} and 10^{-2} mg L^{-1}), with or without filter.**

TEST METHOD	GERMINATION LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	Pr > |T| H0: LSMEAN1=LSMEAN2
No filter	46.0	1.7	0.0001	0.6211
With filter	44.8	1.7	0.0001	

Results (figure 6) showed that covering the extracts with aluminum foil and storing in the refrigerator at 7°C for 20 days did not affect their potency in the parasite stimulation compared with storage for 3 days. Table 4 compares the two storage periods.

Table 4: **Least Square Means of the storage period of aqueous extracts of three cowpea cultivars (Tvx 3236, IT-87D-1951 and Benin Local) at 7°C for 3 and 20 days on response of germination of *Striga hermonthica* seeds**

DAYS STORED	GERMINATION LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	Pr > |T| H0: LSMEAN1=LSMEAN2
3	33.0	1.2	0.0001	0.1565
20	30.5	1.2	0.0001	
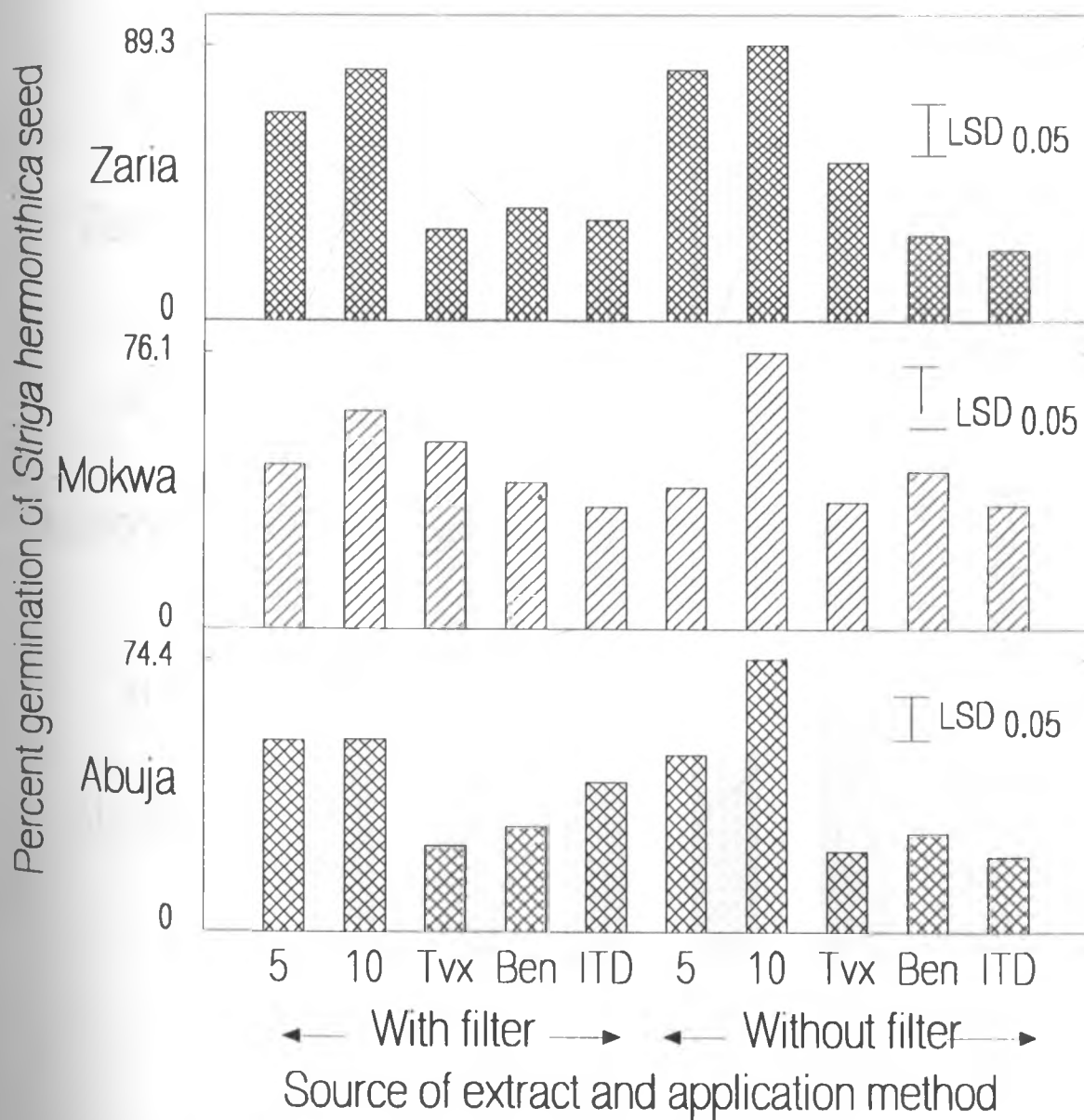
Fig. 5: Germination of *S. hermonthica* seeds in response to aqueous extracts from cowpea cultivars (Tvx-3236, Benin Local and IT-87D-1951) and to GR 24 (5 and 10 (X 10^3mgL^{-1}), applied on or without filter
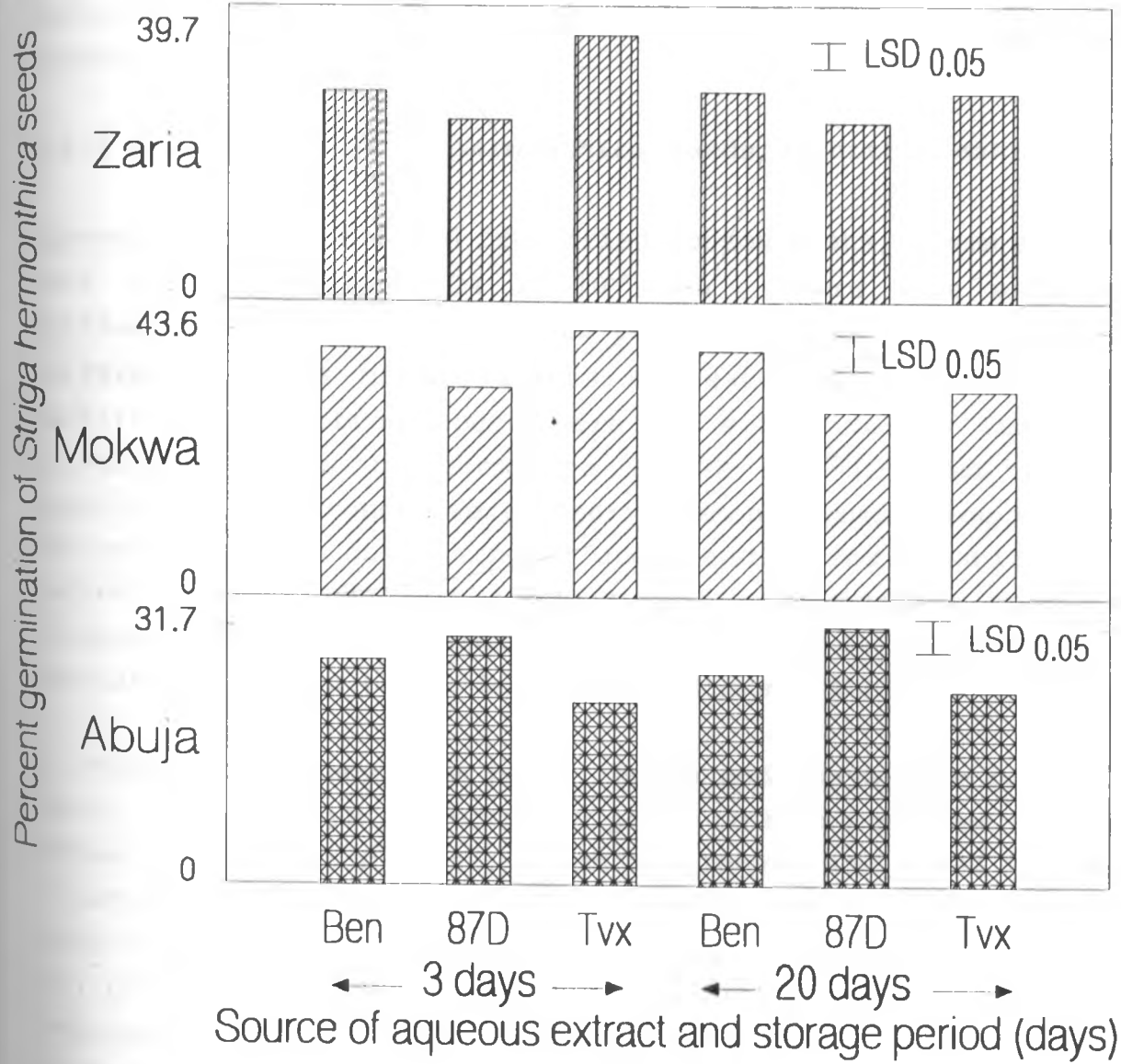
Fig. 6: Germination of *S. hermonthica* seeds in response to aqueous extracts from cowpea cultivars (Benin Local, IT-87D-1951 and Tvx-3236) after being stored at 7⁰ C for 20 days

Relative germination percentages of *S. hermonthica* seed populations induced by different dilutions of aqueous cowpea extracts compared to GR 24 are shown in figure 7. Increasing the concentration of the extracts from 2.5 to 6.3 mg plant tissue/ml of water increased relative percent germination of *S. hermonthica* to 111, 109, and 96 percent for Abuja, Zaria and Mokwa seed populations respectively. Below and above the optimal concentration of the cowpea extracts, relative percent germination of the parasite seeds decreased. The radicles of germinating *S. hermonthica* seeds were shortest at the highest concentration.

1:3:3 Germination of *S. hermonthica* seeds by cotton extracts

Aqueous extracts isolated from dried cotton roots, stems and leaves were compared with GR 24 at 10^{-2} mgL^{-1}. There were significant differences among *S. hermonthica* populations in their response to different sources of stimulants and extracts. Aqueous extracts isolated from cotton roots were as effective in inducing *S. hermonthica* seeds to germinate as GR 24, 10^{-2} mgL^{-1}. However significant differences were observed among germination induced by the extracts from cotton roots, stems and leaves. Distilled water control induced negligible amounts of the parasite germination (figure 8). Inducible germination percent of *S. hermonthica* seed populations was in the order Zaria > Mokwa > Abuja.

S. hermonthica seeds from Zaria, Mokwa and Abuja were conditioned for 4, 7 and 11 days respectively prior to being exposed to aqueous extracts from cotton roots, stems and leaves and to GR 24, 10^{-2} mg L^{-1} and distilled water. Results (table 5) indicate that seeds from Zaria had the highest response to aqueous cotton extracts and GR 24, 10^{-2} mg L^{-1}. There were no significant differences between responses shown by seeds from Mokwa and Abuja to the extracts. Extracts from cotton roots induced the highest germination percent of the parasite seeds whereas extracts from cotton stems performed
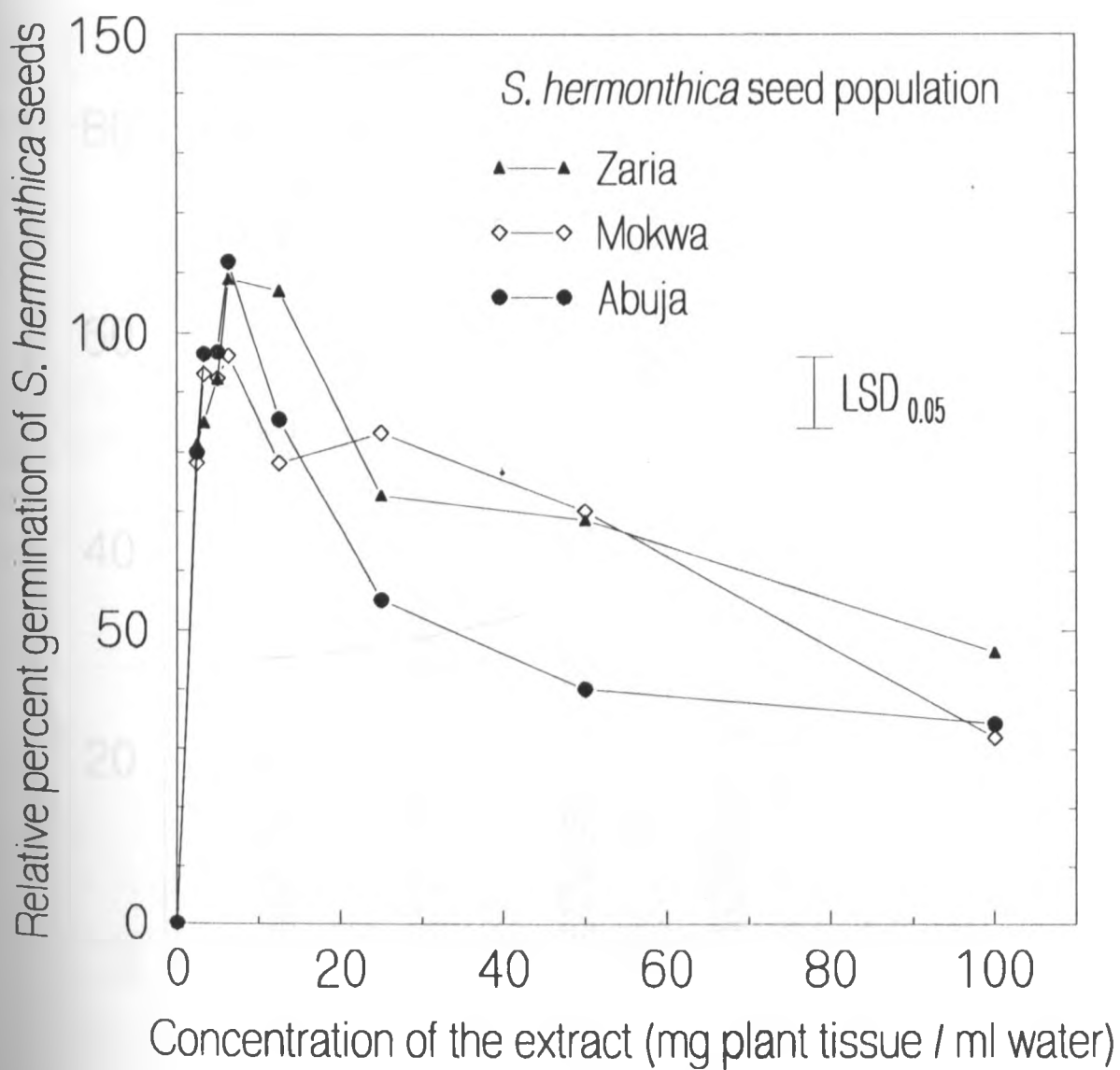
Fig. 7: Germination of three populations of *Striga hermonthica* seeds in response to varying concentrations of aqueous extracts of dried roots and shoots of cowpea cultivar (Tvx 3236).
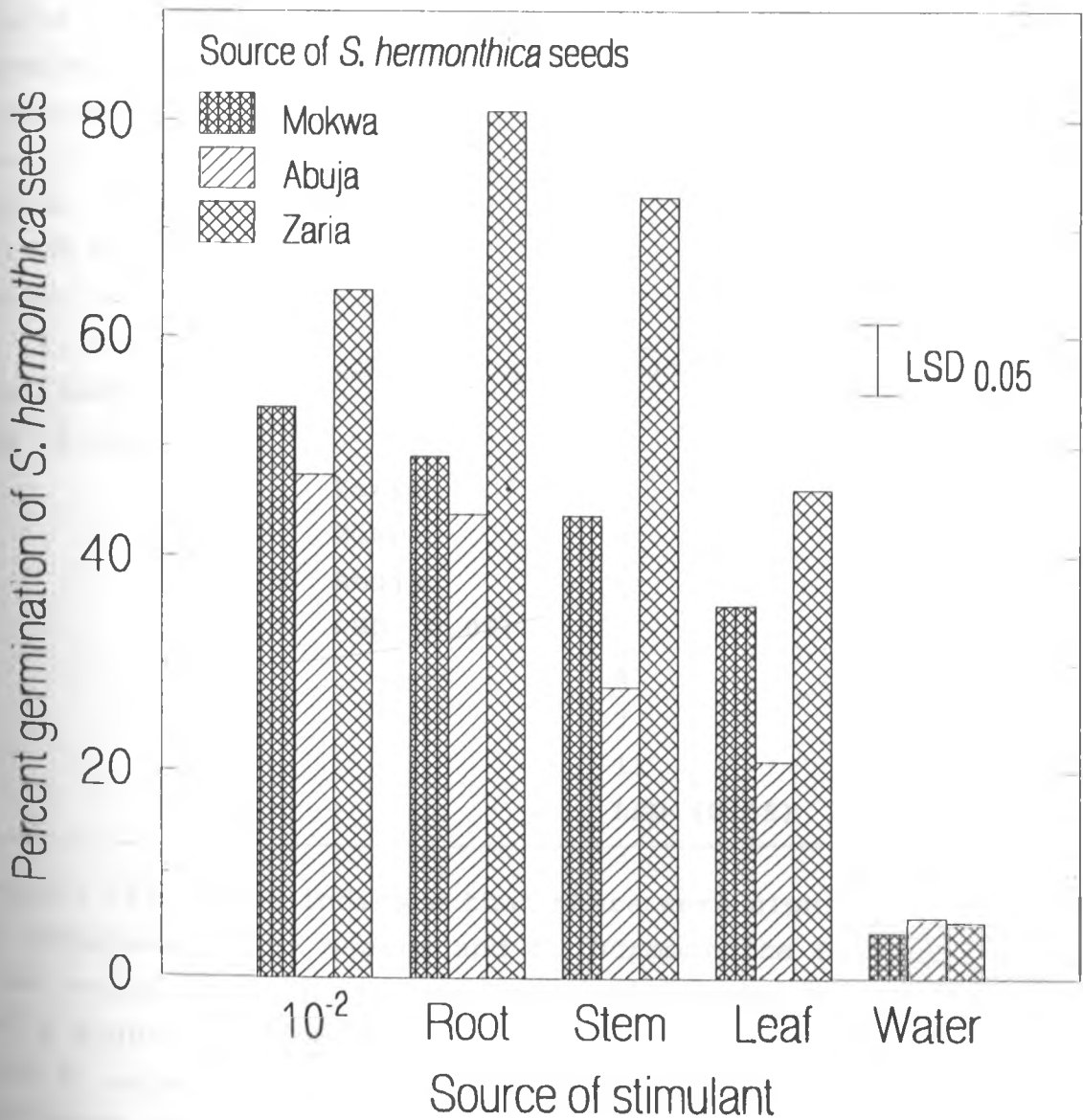
Fig. 8: Germination of three populations of *Striga hermonthica* seeds in response to aqueous extracts from dried cotton roots, stems and leaves (12.5 mg plant tissue / ml water and to strigol analog GR 24 (mgL^{-1})

as well as GR 24, 10^{-2} mg L^{-1}. The most optimal conditioning period was 11 days.

Table 5: Effects of source of stimulant, *S. hermonthica* seed and days of conditioning on germination of the parasite seeds by aqueous extracts from cotton plant parts and GR 24.

Source of Germination stimulant	Source of seed	Conditioning days	percent
Cotton root	-	-	58.6
cotton shoot	-	-	47.8
cotton leaves	-	-	32.5
GR 24, 10^{-2} mgL^{-1}	-	-	49.1
Distilled water	-	-	3.2
LSD (0.05)	-	-	**3.75**
	Zaria	-	52.2
	Mokwa	-	32.6
	Abuja	-	29.9
	LSD (0.05)	-	**2.90**
		4	36.9
		7	37.6
		11	40.2
		LSD (0.05)	**2.90**

Results (figure 9) indicate that there were significant differences in responses of *S. aspera* and *S. hermonthica* to aqueous extracts from cotton plant parts and their mixtures. *S. hermonthica* seeds had a higher germination response to aqueous extracts from cotton than *S. aspera* seeds. In both parasites, mixtures of the extracts depressed seed germination below the average percent germination induced by individual stimulants in the mixture. Mixtures of aqueous extracts from cotton root and leaf and stem and leaf
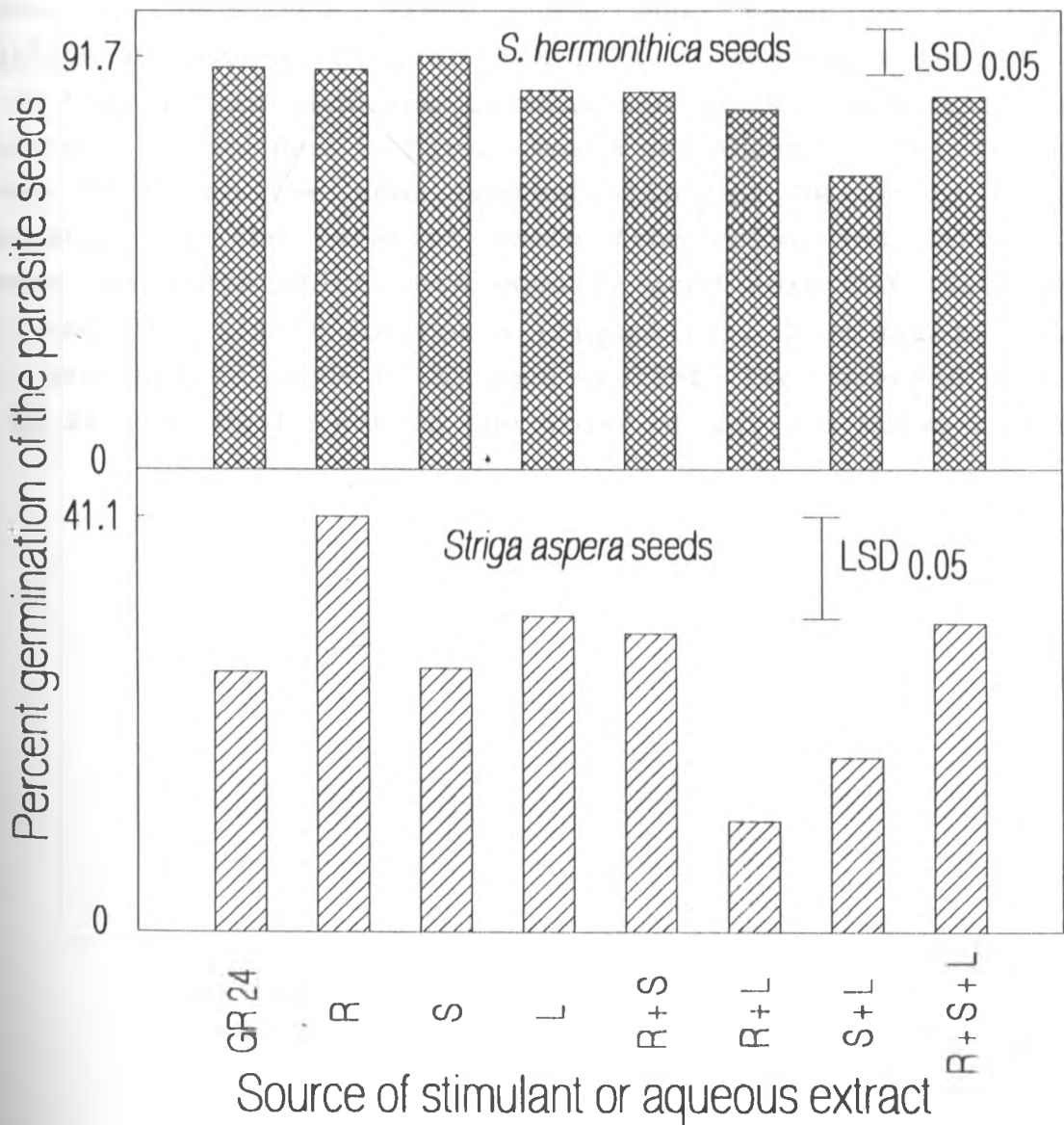
Fig. 9: Germination of the parasite seeds in response to GR 24 (10^{-2}) mgL^{-1} and to aqueous extracts from cotton plant parts and their mixtures

significantly depressed germination of seeds of the two *Striga* species. However, some of the extracts and their mixtures were as effective in inducing germination of the seeds of both parasites as GR 24 at 10^{-2} mg L^{-1}.

1:3:4 Germination of *S. hermonthica* seeds by soybean extracts

Aqueous extracts from fresh stems and leaves of 10 day old seedlings of soybean cultivar, TGX-1674-1F, were as effective as GR 24, 10^{-3} mg L^{-1} in inducing germination of *S. hermonthica* seeds collected from sorghum in Zaria, Mokwa and Abuja in 1991. TGX-1681-3F was equal to the same concentration of GR 24 in terms of germination of the parasite seeds from Mokwa and Zaria. Other soybean cultivars which were equally good were TGX-1660-18F for Zaria and TGX-1674-8F for Mokwa (figure 10). Germination response of *S. hermonthica* seeds to aqueous extracts from soybean cultivars and GR 24, 10^{-3} mg L^{-1} was in the order of Zaria > Mokwa > Abuja.
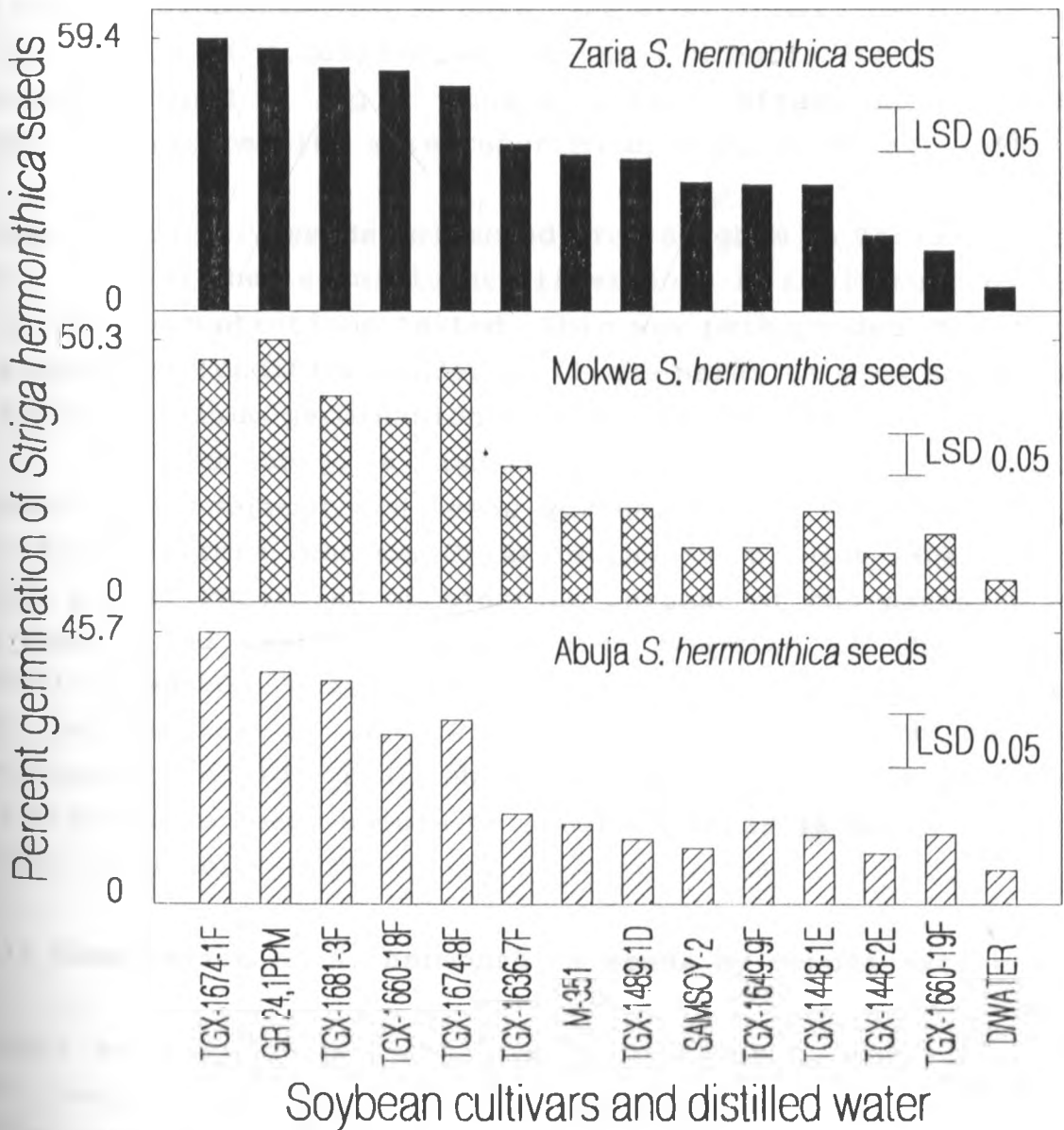
Fig. 10: Germination of *S. hermonthica* seeds in response to aqueous extract (25mg plant tissue / ml water) from 10 day old fresh shoots of soybea

1:4 DISCUSSION

1:4:1 Germination of *Striga hermonthica* seeds by GR 24

Exposing *S. hermonthica* seeds to a suitable concentration of GR 24 after conditioning in the dark at 28°C for 4 to 30 days gives a high germination response. There is interaction between conditioning period and GR concentration. The parasite seeds should not be conditioned beyond 30 days. The drop in germination percent, at all levels of conditioning, as the concentration of GR 24 was increased beyond 10^{-2} mg L^{-1} suggest a toxic effect on the parasite seeds. Similar results were reported by Babiker *et al*, (1991).

Striga hermonthica seeds collected from sorghum in Zaria, Mokwa and Abuja, in 1991, had significant differences in their response to GR 24 at the concentrations tested. This was perhaps due to different environmental conditions under which the parasite grew and matured. To achieve maximum germination response of the parasite seeds to GR 24 in the laboratory, a concentration of 10^{-2} mg L^{-1} should be applied on *S. hermonthica* seeds sprinkled on 8 mm diameter glass microfibre filters (Whatman GF/A), arranged on moist (Whatman No.1) filter paper. Recording germination percent of the parasite seeds was easier and faster with the disc compared to the multiwell incubation method. Where multiwell method of incubation is used to test germination of *Striga hermonthica* seeds with GR 24, a concentration of 10^{-2} mg L^{-1} should be used and percent germination of the parasite seeds should be recorded after 48 hours to achieve maximum results.

1:4:2 Germination of *S. hermonthica* seeds by cowpea extracts

Aqueous extracts from three cowpea cultivars (Benin Local, Tvx 3236, and IT 87D 1951) contain germination stimulants for *S. hermonthica*. This is shown by the level of germination induced by extracts on three populations of the parasite seeds. Unlike GR 24

which stimulated the highest germination in *S. hermonthica* seeds from Zaria, aqueous cowpea extracts induced highest germination in *S. hermonthica* seeds from Mokwa. This suggests interaction between the source of the stimulants and the parasite seed populations. The differences in response of the various parasite seeds to aqueous extracts are perhaps due to ecological differences under which the parasite seeds developed or differences in physiological strains.

Results have indicated that the extracts can be tested on discs containing *S. hermonthica* seeds without first putting two filter papers in the petri-dish, without compromising the outcome of the results. The amount of extract applied is half the amount required when filter paper is placed in the petri-dish before application of the extract. This would be important where the amount of extract is limited and eliminate the need of using the filter papers especially where they are not available. However care is required when applying the extract without the use of filter paper to avoid washing the parasite seeds.

Storing the extracts at 7°C, covered with aluminum foil would greatly eliminate the need to prepare fresh extracts every time. Fungal contamination of the extract was greatly eliminated under these conditions and the potency of the extracts were maintained.

Germination of *S. hermonthica* seeds in response to increased concentrations of aqueous cowpea extracts does not follow a linear response. Results indicate that germination of the parasite seeds increases as the concentration of the extracts increase to an optimum beyond which germination decreases with further increase in concentration. This perhaps suggest the presence of both inhibitory and stimulatory substances of *S. hermonthica* in aqueous extracts of cowpea. Dilution of the extract probably tipped the balance in favor of the stimulant(s).

The existence of *S. hermonthica* germination inhibitors in aqueous cowpea extracts opens up the interesting possibility of extraction, identification and use of such compounds to stop the germination of the parasite seeds in the soil as means of control. Similar depressive effects at high concentration of root extract of *Vicia faba* were reported in the germination of *Orobanche minor* and *O. crenata* (Brown et al, 1951; Mallet, 1973; Whitney, 1979).

It was observed, from the results, that the concentration of aqueous germination stimulants for *S. hermonthica* seeds isolated from mature cotton plant increase from the leaves to the stems and reach maximum in the roots. The concentration of aqueous stimulants from the roots of cotton is equivalent to that of 10^{-2} mg L^{-1} of GR 24 in terms of percent of the induced germination of *S. hermonthica* seed populations. Germination stimulants of *S. hermonthica* seeds are not restricted to the living tissues of root of crop seedlings. Germination stimulants in aqueous extracts were found distributed in the roots, stems and leaves of mature cotton and the whole plant of three cowpea cultivars in sufficient concentrations to cause high germination of the parasite seeds.

1:4:3 Germination of *S. hermonthica* seeds by cotton extracts

Aqueous extracts from cotton root, stems and leaves contain high concentrations of germination stimulants for *S. aspera* and *S. hermonthica* seeds. Mixtures of two or more of these aqueous extracts are antagonistic to germination of the parasite seeds. Perhaps the interaction of germination inhibitors and stimulants is a contributing factor to this antagonism. From this experiment cotton is a trap crop for both *S. aspera* and *S. hermonthica* and growing it in fields infested with the two parasite seeds would enhance their death through germination of the seeds in the absence of the host.

In order to achieve maximum germination response of *S. hermonthica* seeds to the extracts from cotton roots, stems and leaves and to strigol analog, GR 24 at 10^{-2} mg L^{-1}, the minimum conditioning period should between 7 to 21 days in the dark at 28°C.

1:4:4 Germination of *S. hermonthica* seeds by soybean extracts

Mercerating fresh stems and leaves of 10 day old soybean seedlings in distilled water and application of the extract on *S. hermonthica* seeds conditioned in the dark for 11 days is a quick and cheap method of cultivar selection for high parasite seed germination stimulation in the laboratory. The cultivars with high germination stimulants can then be planted in infested fields in rotation with *S. hermonthica* susceptible cereals to reduce infestation and parasitism the following season. Such rotations would be more effective in Zaria, where TGX-1674-1F, TGX-1681-3F and TGX-1660-18F induced germination of *S. hermonthica* seeds by more than 50 percent. Inclusion of the roots would greatly improve stimulation since the parasite germination stimulants are more concentrated in the roots, followed by stems and the leaves.

CHAPER 2: PARTITIONING AND EFFECTIVENESS OF *STRIGA HERMONTHICA* SEED GERMINATION STIMULANTS IN COTTON AND COWPEA AT DIFFERENT STAGES OF CROP GROWTH.

ABSTRACT

Seed germination of the hemiparasite angiosperm *Striga hermonthica* is unique in so far as it is triggered by compounds released from the roots of hosts and non host plants. Results indicated that relative percent germination of *S. hermonthica* seeds by aqueous extracts from cotton roots and stems and cowpea roots and leaves decreased with increasing growth stages of the crops. Aqueous extract from cotton leaves and cowpea stems increased the parasite seed germination to a maximum, at 40 days of cotton and cowpea growth, thereafter decreasing with increasing age of the crops. Extracts from cotton bolls at 100 days of growth induced 12 relative percent germination of the parasite seeds, while that from cowpea pods achieved 9.9 and 9.7 relative percent gemination at 70 and 100 days of growth respectively. No germination of the parasite seeds was achieved with aqueous extracts from cotton and cowpea seeds.

Aqueous extracts from cotton was found to be better in inducing *S. hermonthica* seed germination than that from cowpea at corresponding stages of growth. Extracts from cotton roots and stems were as effective in inducing the parasite seed germination as GR 24, 10^{-2} mg L^{-1}.

Significant differences (P=0.05) were recorded in germination of *Striga hermonthica* seeds by dichloromethane extracts from 70 days old cotton and cowpea plant parts at different concentrations. Significant interactions were observed between sources of stimulants and their concentrations in dimethyl sulfoxide. Relative percent germination of *S. hermonthica* seeds by dichloromethane extract from cotton and cowpea roots increased with increasing

concentration upto a maximum, 0.5 percent for cotton and 1 percent for cowpea, then declined. Germination activity of extracts of cotton leaves and cowpea stems decreased with increasing concentration whereas those from cotton bolls, cowpea leaves and pods and dimethylsulfoxide increased with increasing concentration. Very little germination activity was recorded with extracts from cowpea seed and cotton stems.

Dichloromethane soluble extracts from cotton and cowpea plant parts have several compounds as showed by the active bands (Rfs) obtained from Thin Layer Chromatography (TLC). Even cowpea seeds which did not show appreciable germination activity on *S. hermonthica* seeds had similar compounds as others with Rfs (0.61, 0.89 and 0.97). *S. hermonthica* seeds showed similar compounds to those found in the extracts. DCM soluble extracts of cotton and cowpea plant parts of different ages were separated by thin layer chromatography. Results showed that the extracts contained several similar compounds as shown by position of relative fronts. DCM extracts of *S. hermonthica* seeds contained similar compounds.

Germination of *S. hermonthica* seeds by DCM soluble extracts of cotton and cowpea roots increased with increasing concentration from 0.1 to 0.5 % and 0.1 to 1.0 % for cotton and cowpea respectively and then declined with further increase in concentration. Extracts from cotton leaves and cowpea stems behaved differently with increase in concentration to 1.0%, from extracts from cotton bolls, cowpea leaves and pods.

Screenhouse experiments to determine the effects of previous season cowpea and cotton crops on *S. hermonthica* parasitism on maize indicated that growing cotton (Abuja Local) or cowpea (Tvx 3236) in *S. hermonthica* infested soil the previous season significantly reduced parasitism on maize planted the following season and increased yields. Unemerged attached, emerged, total attached *S. hermonthica* and root dry weight were positively correlated with

symptom severity on maize. All parameters were negatively correlated with grain weight, and all, except stem dry weight were negatively correlated with harvest index.

Unemerged attached, emerged and total attached parasites on each maize plant decreased with increasing length of cotton or cowpea growth the previous season. No significant advantage was obtained by addition of fresh cotton or cowpea mulch.

2:1 INTRODUCTION

Germination of *Striga hermonthica* seeds depend on after ripening, conditioning in a warm moist environment for several days and subsequent exposure to an exogenous germination stimulant, usually produced as exudates from the roots of a number of host and non-host plants (Andrews, 1945; Smalling *et al*, 1991 and Mangnus *et al*, 1992). Different host plants produce different mixtures of stimulants, as demonstrated by chromatographic investigation (Visser and Botha, 1974).

In the Sudan, Andrews (1945) from pot experiments, and later Wilson-Jones (1953) and Last (1961) from field experiments, have found that the sowing of groundnut, cowpea, and dolichos bean in a *S. hermonthica* infested soil did reduce a large proportion of the parasite seed. In India, in a pot experiment , Yaduraju and Hosmani (1979) reported that all trap crops including cowpeas, groundnuts, and linseed decreased the incidence of *S. asiatica* on the succeeding crop with cowpea and groundnut being the most effective.

In severely infested fields, one to several years may elapse before the *Striga* spp. population is reduced to non damaging levels. Where groundnut (*Arachis hypogaea* L.), field beans, sunhemp (*Crotalaria juncea* L.) and buckwheat (*Fagopyrum esculentum* L.) were grown previous to corn (*Zea mays*) crop, Rose and Rochrie, 1941, showed that ''there was good reason for suspecting that these trap crops had actually destroyed much of the *Striga* seed by causing it to germinate '', following germination of these seeds in the absence of a host. The objective of this study was to determine partitioning and effectiveness of germination stimulants from cotton and cowpea plants on germination of *Striga hermonthica* seeds.

2:2 MATERIALS AND METHODS

2:2:1 Partitioning of germination stimulants and crop rotation

These studies were conducted on the soil floor of a screenhouse at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, in 1993. Ridges, 1.75 m long spaced 75 cm apart were infested with 24,000 clean germinable *S. hermonthica* seeds collected from sorghum in Zaria, Nigeria, in 1991. The parasite seeds were mixed with fine sand and distributed along the length of the ridges to give uniform distribution. The *S. hermonthica* seed/sand mixture was covered lightly with soil and watered once daily for 7 days before the cotton and cowpea were planted. The experiment was completely randomized and treatments were replicated four times. Cotton, cv. Abuja Local, and cowpea, cv. Tvx 3236, were previously selected in the laboratory for high *S. hermonthica* seed germination ability.

Seeds of these cultivars were planted 25 cm apart along the ridges for 10, 40, 70, and 100 days, after emergence, respectively. Four plants were grown in the 10 day treatment, and one plant was grown in each of the other growth period treatments. In addition to these treatments, 1 kg of fresh mulch of cowpea or 2 kg of cotton mulch, after harvesting cotton and cowpea at 100 days, were laid along respective ridges. After each of the growth periods, the crops were uprooted and one plant of susceptible maize cv. 8338-1 was grown in each original planting hole. In the controls, maize was grown in infested soil which was fallow the previous season.

Maize was grown for 12 weeks and then harvested. Counts of emerged *S. hermonthica* plants were recorded fortnightly until harvest. Symptom scores of the parasite damage on maize were recorded 8 weeks after planting on a scale of 0=no damage to 9=heavy damage. Maize was harvested by cutting the plants at ground level. The maize roots were uprooted with a hoe and submerged in a bucket of

water to remove the soil. Unemerged *S. hermonthica* attached to the host roots were counted in each treatment. Dry biomass of maize shoots, roots and grain yields were recorded after drying in the oven at 60°C for 72 hours. Harvest index (grain yield/total biomass) was calculated for each treatment. Treatments were statistically compared. *S. hermonthica* and maize growth parameters were also analysed by correlation.

At each date of harvesting cotton and cowpea, roots were separated from stems and leaves. At maturity pods and seeds were separated for cowpea and bolls for cotton. These were dried in the oven at 60°C for 3 days and ground to pass through 2 mm sieve. Each sample of 5 g was soaked in 400 ml of water for 1 hour at room temperature. The samples were filtered and 0.13 ml of the extract was applied on each 8 mm disc containing 25-50 conditioned *S. hermonthica* seeds, in a petri-dish, lined with two filter papers. The dishes were sealed with paraffin wax and incubated in the dark at 28C for 48 hours before counting the parasite germinated under a microscope with 20 times magnification.

2:2:2 Preparation of dichloromethane soluble fraction

Samples of 5 g each of dried and ground roots and shoots of each trap crop were weighed in a beaker and labeled. To each sample, 200 ml of dichloromethane was added and stirred. The mixture were covered with a lid and left over night at room temperature. The samples were filtered, bottled and labeled, making sure that no plastic material came into contact with dichloromethane. The extract were evaporated to dryness in a rotary evaporator at 45 degrees centigrade. The residue was dissolved in concentrated DMSO (Dimethylsulfoxide) to enhance solubilization before water was added to give a final concentrations of 0.1, 0.5, 1.0 and 5.0% DMSO V/V (Fischer *et al*, 1990). The same concentrations of DMSO in distilled water was used as controls. These were later tested on germination of *S. hermonthica* seeds.

A streak (15 cm) of crude dichloromethane extract samples from cotton and cowpea plant parts at different ages were applied on silica gel chromatoplates, 2 cm from the base of the plates. The plates were developed by ascending technique with 35% ethyl acetate / 65% hexane (v/v) until the solvent reached about 16 cm from the origin. The spray reagent was a mixture of cobalt chloride hexahydrate / sulphuric acid. The positions of different compounds in each extract were measured from the base of the front, using a ruler. Relative Fronts (Rfs) were calculated as ''the distance moved by solute/the distance moved by the solvent''.

2:3 RESULTS

2:3:1 Germination stimulation by dichloromethane soluble extracts.

Significant differences (P=0.05) were recorded in germination of *Striga hermonthica* seeds by dichloromethane extracts from 70 days old cotton and cowpea plant parts at different concentrations (figures 1 and 2). Significant interactions were observed between sources of stimulants and their concentrations in dimethyl sulfoxide. Relative percent germination of *S. hermonthica* seeds by dichloromethane extract from cotton and cowpea roots increased with increasing concentration upto a maximum, 0.5 percent for cotton and 1 percent for cowpea, then declined. Germination activity of extracts of cotton leaves and cowpea stems decreased with increasing concentration whereas those from cotton bolls, cowpea leaves and pods and dimethylsulfoxide increased with increasing concentration. Very little germination activity was recorded with extracts from cowpea seed and cotton stems.

Dichloromethane soluble extracts from cotton and cowpea plant parts have several compounds as showed by the active bands (Rfs) obtained from Thin Layer Chromatography (TLC) in table 1. Even cowpea seeds which did not show appreciable germination activity on *S. hermonthica* seeds had similar compounds as others with Rfs (0.61, 0.89 and 0.97). *S. hermonthica* seeds showed similar compounds to those found in the extracts.
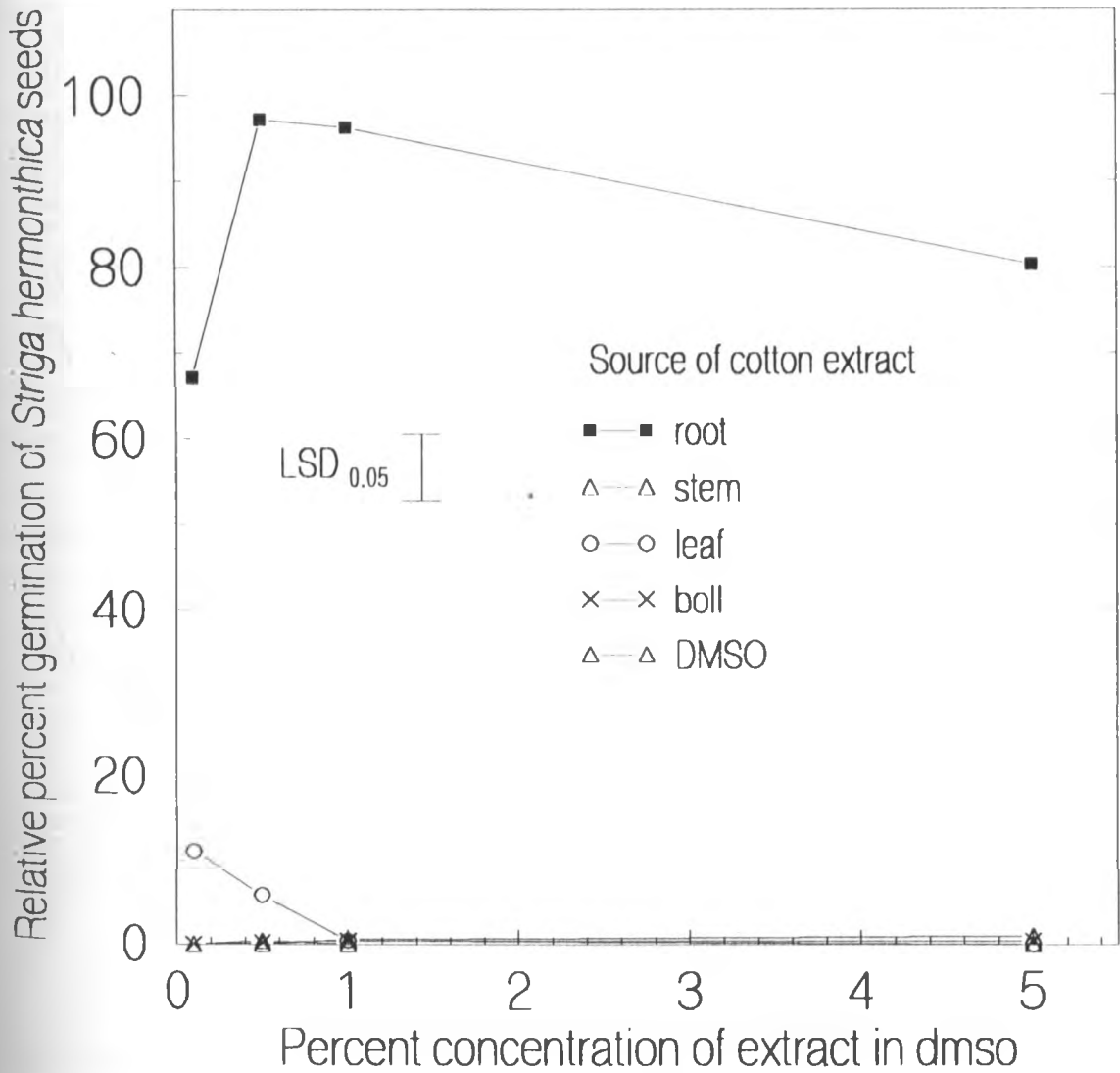
Fig. 1: Relative percent germination of *S. hermonthica* seeds in response to dichloromethane soluble extract residues from cotton roots, stems, leaves and bolls redissolved in various concentrations of dimethyl sulfoxide
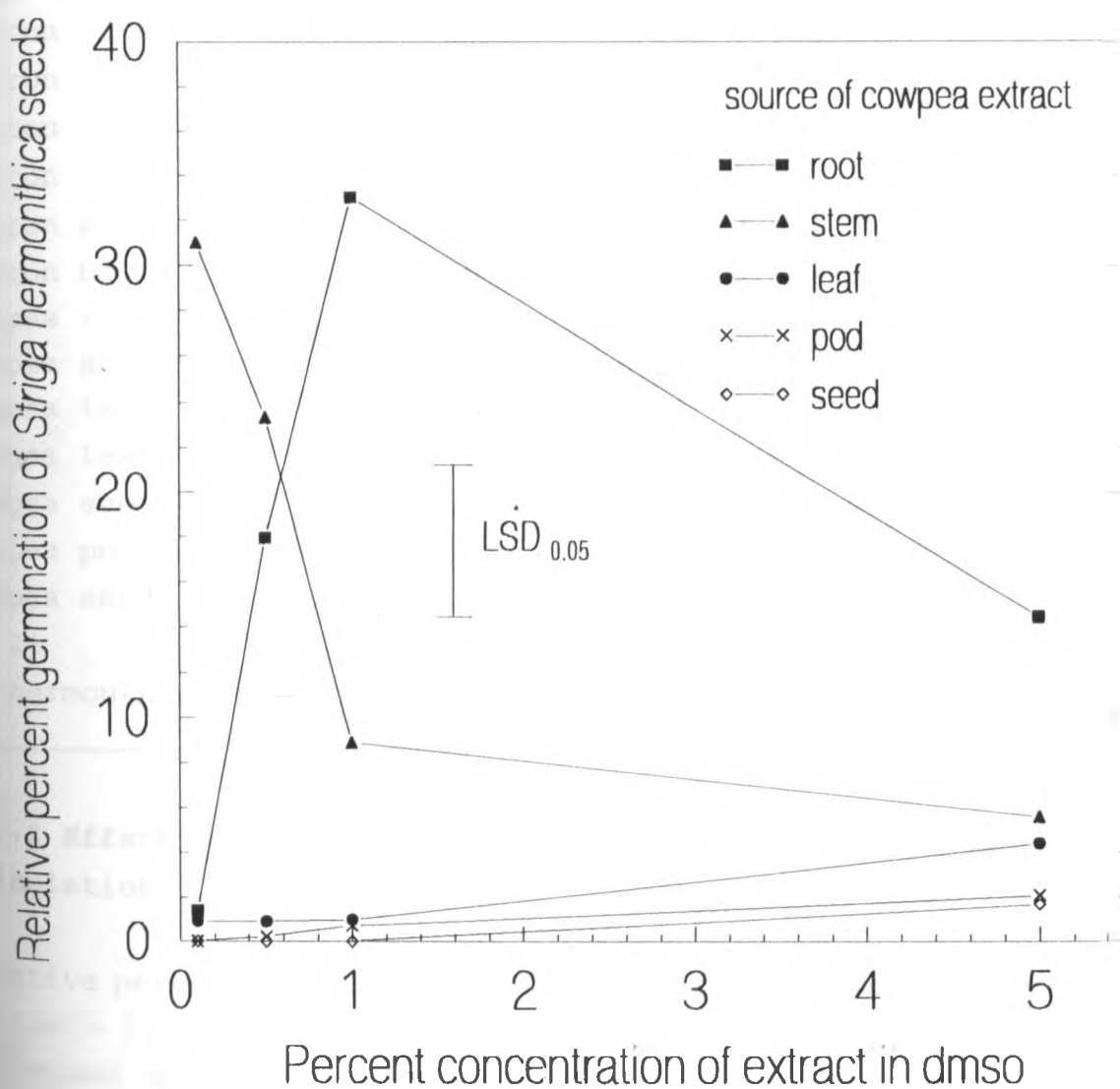
Fig. 2: Relative percent germination of *S. hermonthica* seeds induced by dichloromethane soluble extract residues from cowpea roots, stems, leaves, pods, and seeds in various concentrations of dimethyl sulfoxid

Table 1: Thin Layer Chromatography of dichloromethane extracts of cotton (Abuja Local) and cowpea (Tvx-3236) at different stages of growth.

Source of extract	age (days)	Relative front (Rf)
Cotton root	10	0.90
Cotton root	70	0.06, 0.71
Cotton stem	10	0.76, 0.85, 0.97
cotton stem	70	0.23, 0.32
Cotton leaf	10	0.76, 0.85, 0.97
Cotton leaf	70	0.65, 0.71
Cotton shoot	10	0.76, 0.85, 0.97
cotton bolls	70	0.71, 0.81, 0.90
Cowpea root	70	0.48
Cowpea stem	70	0.23, 0.42, 0.71
Cowpea leaf	10	0.06, 0.71
Cowpea leaf	70	0.76, 0.85
Cowpea shoot	10	0.76, 0.85, 0.97
cowpea pod	70	0.16, 0.61, 0.90, 0.97
cowpea seed	70	0.61, 0.89, 0.97
GR 24	-	0.28, 0.36,
S. hermonthica seeds	-	0.61, 0.78, 0.85, 0.94

2:3:2 Effect of age of cotton and cowpea on germination stimulation

Relative percent germination of *Striga hermonthica* seeds by aqueous extracts from cotton roots and stems and cowpea roots and leaves decreased with increasing growth stages of the crops. Aqueous extract from cotton leaves and cowpea stems increased the parasite seed germination to a maximum, at 40 days of cotton and cowpea growth, thereafter decreasing with increasing age of the crops (figures 3 and 4). Extracts from cotton bolls at 100 days of growth
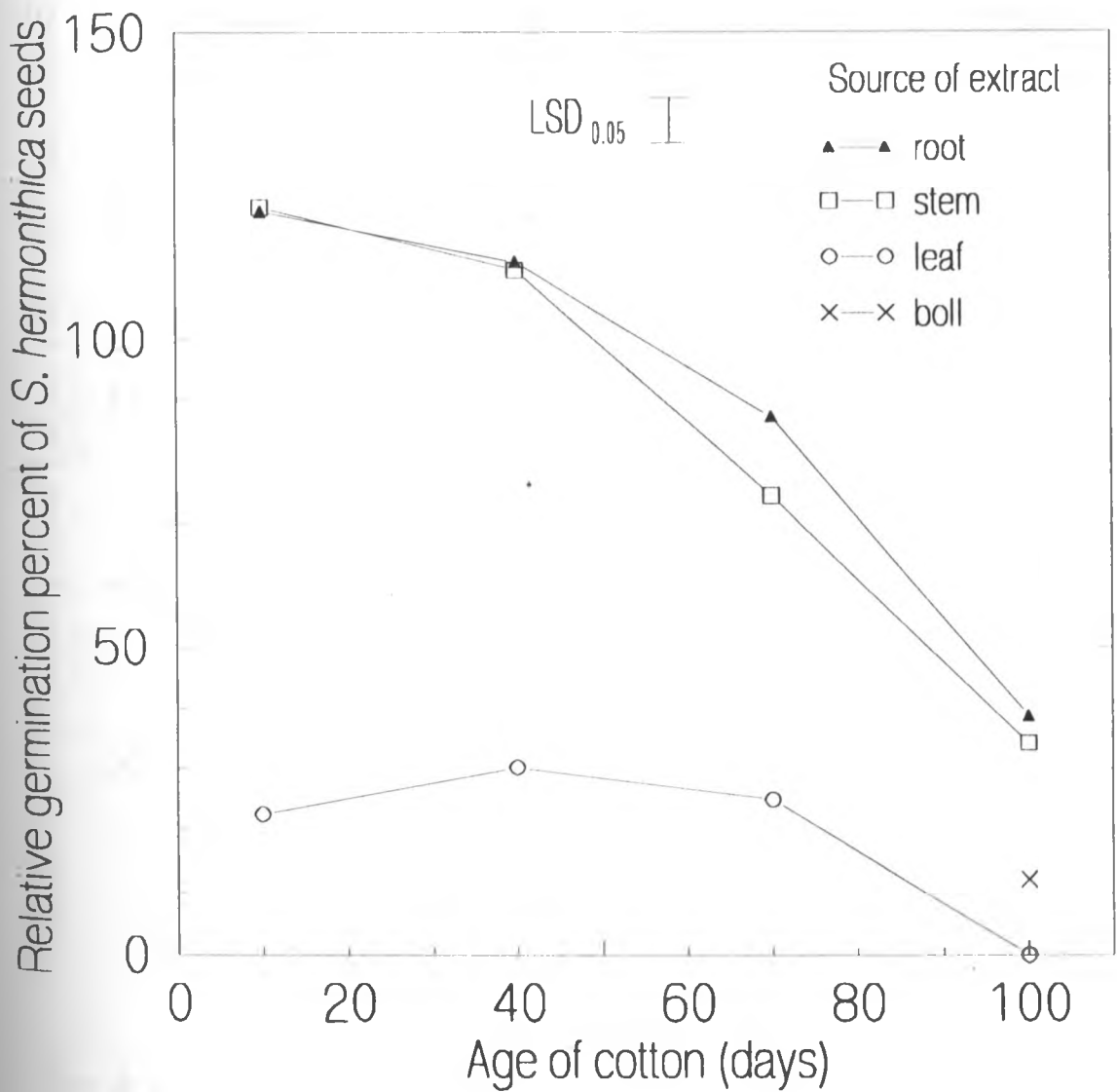
Fig. 3: Germination of *Striga hermonthica* seeds in response to aqueous extracts from dried roots, stems, leaves and bolls (12.5mg plant tissue / ml water of cotton cultivar (Abuja Local) at different ages of plant growth
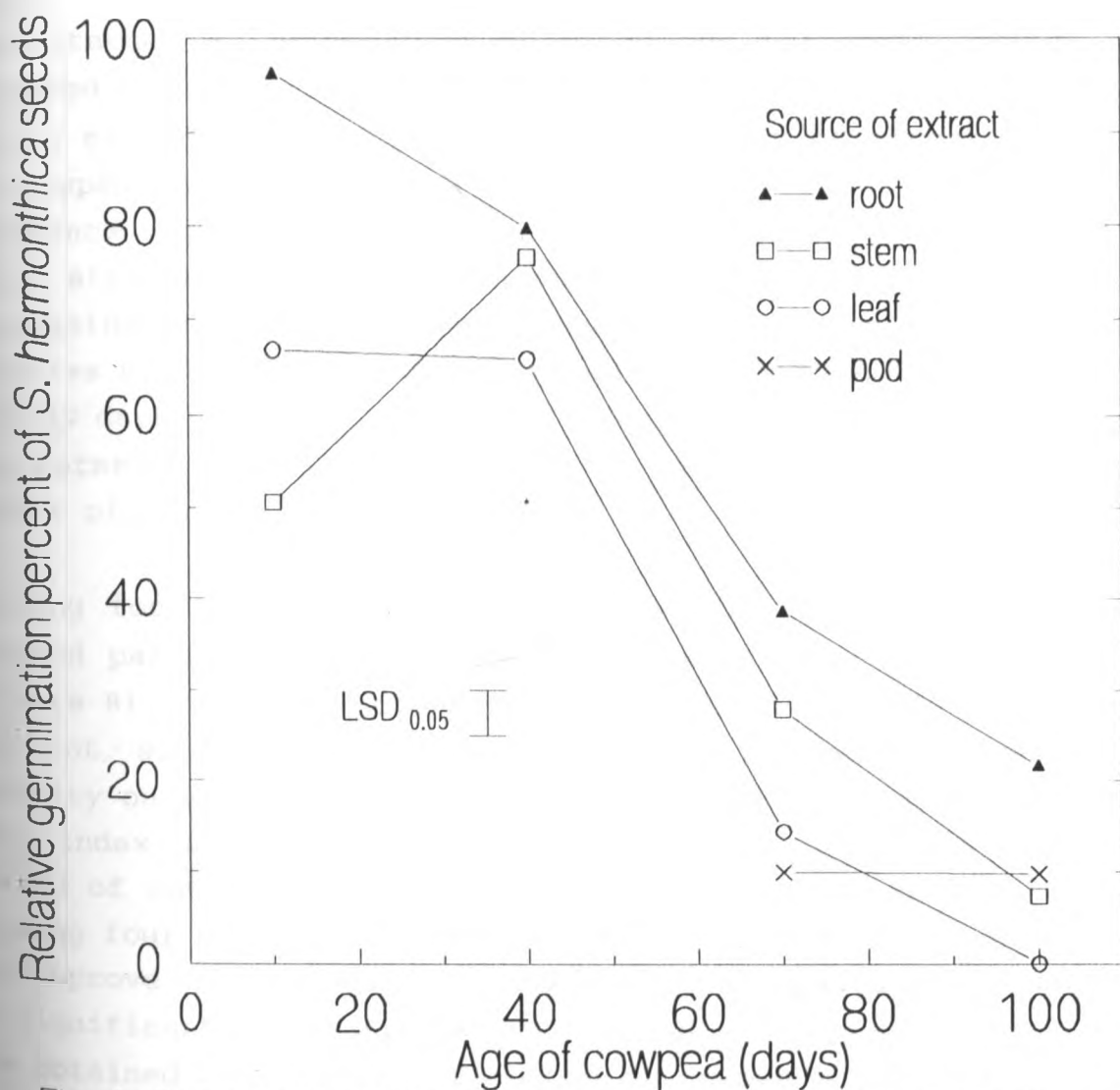
Fig. 4: Germination of *Striga hermonthica* seeds in response to aqueous extracts (12.5mg plant tissue / ml water) from dried roots, stems, leaves and pods of cowpea cultivar (Tvx 3236) at different ages of plant growth.

induced 12 relative percent germination of the parasite seeds, while that from cowpea pods achieved 9.9 and 9.7 relative percent gemination at 70 and 100 days of growth respectively. No germination of the parasite seeds was achieved with aqueous extracts from cotton and cowpea seeds.

Results indicate that growing four plants of cotton, cv. Abuja Local, in infested soil for 10 days reduced the number of emerged and attached *S. hermonthica* on maize the following season when compared with the control. Four plants of cowpea grown for 10 days had no effect on these parameters. One plant of either the cotton or cowpea cultivar, grown for 40 days, significantly reduced emergence and attachment. The number of unemerged, emerged and total attached *S. hermonthica* on each maize plant decreased with increasing period of cotton or cowpea growth the previous season (figures 5, 6 and 7). However, growing the non-host crops for more than 40 days did not significantly further reduce the attachment of the parasite on maize. Addition of mulch had no effect on the number of parasites attached. '

Growing four cotton or cowpea plants for 10 days significantly reduced parasite symptom severity on maize the following season (figure 8). Thinning to one plant and growing for 40 days or more did not significantly further reduce *S. hermonthica* symptom severity on maize. Maize grain yield per plant (figure 9) and harvest index (figure 10) increased significantly with increasing period of cotton and cowpea growth the previous season. However, growing four plants of cowpea for 10 days the previous season, did not improve maize yield or harvest index compared to the control. No significant advantage on grain yield or harvest index of maize was obtained by addition of cotton or cowpea mulch.
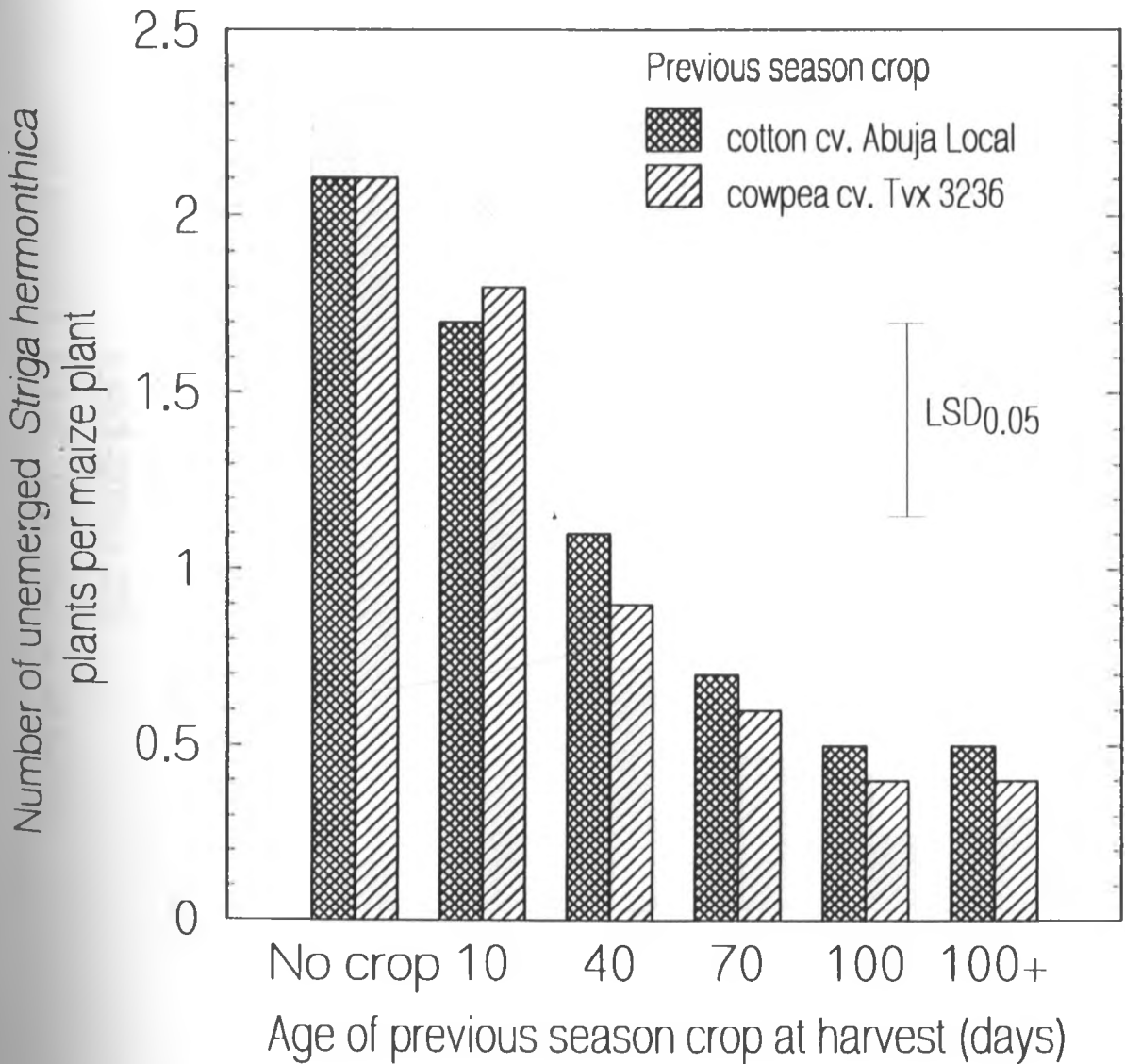
Fig. 5: Number of unemerged *Striga hermonthica* per maize plant.
A ' + ' denotes that 1 Kg or 2 Kg of fresh mulch from cowpea or cotton,
respectively, was added, per ridge, before maize was planted.
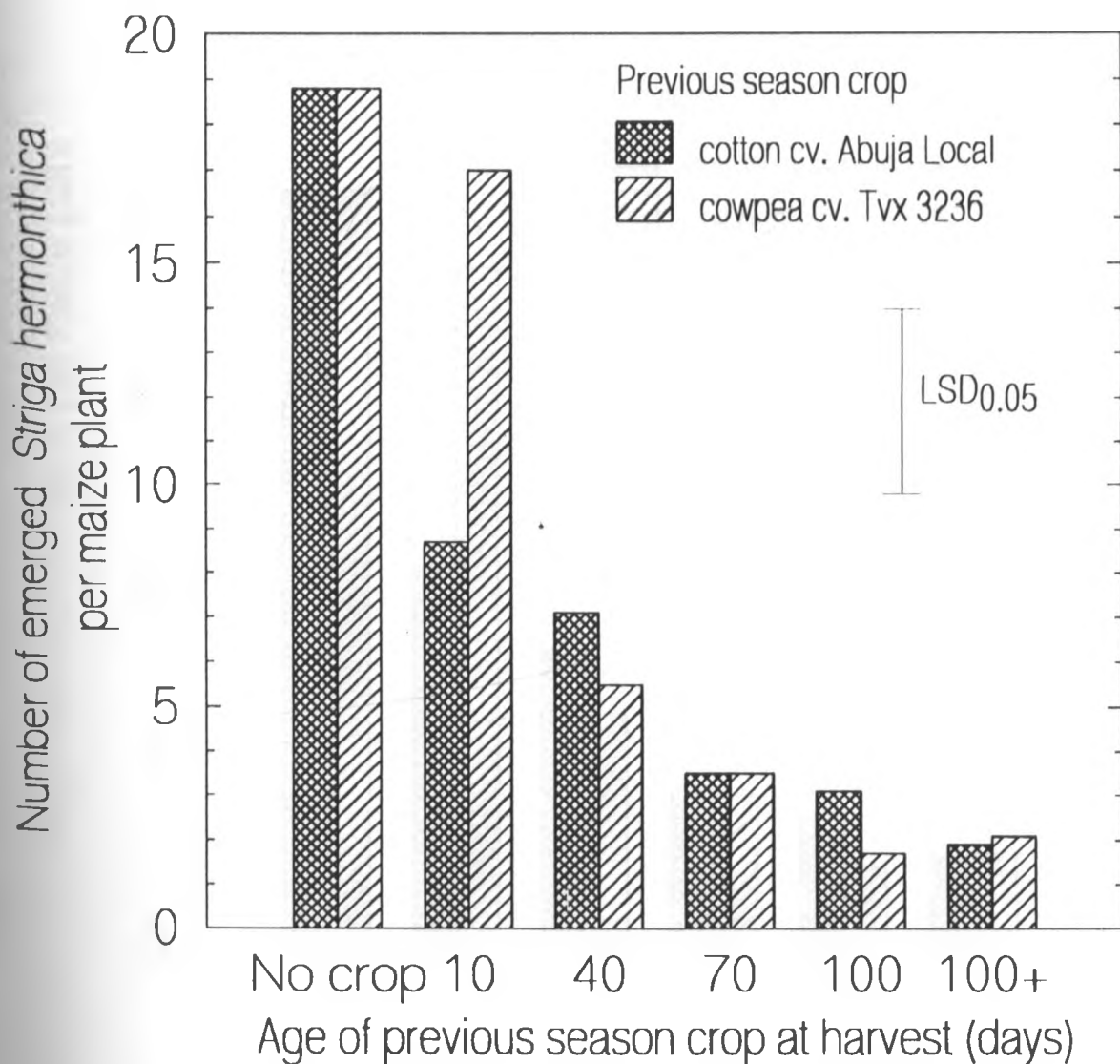
Fig. 6: Number of emerged *Striga hermonthica* plants per maize root. A ' + ' denotes that 1 Kg or 2 Kg of fresh mulch from cowpea or cotton, respectively, was added, per ridge, before maize was planted.
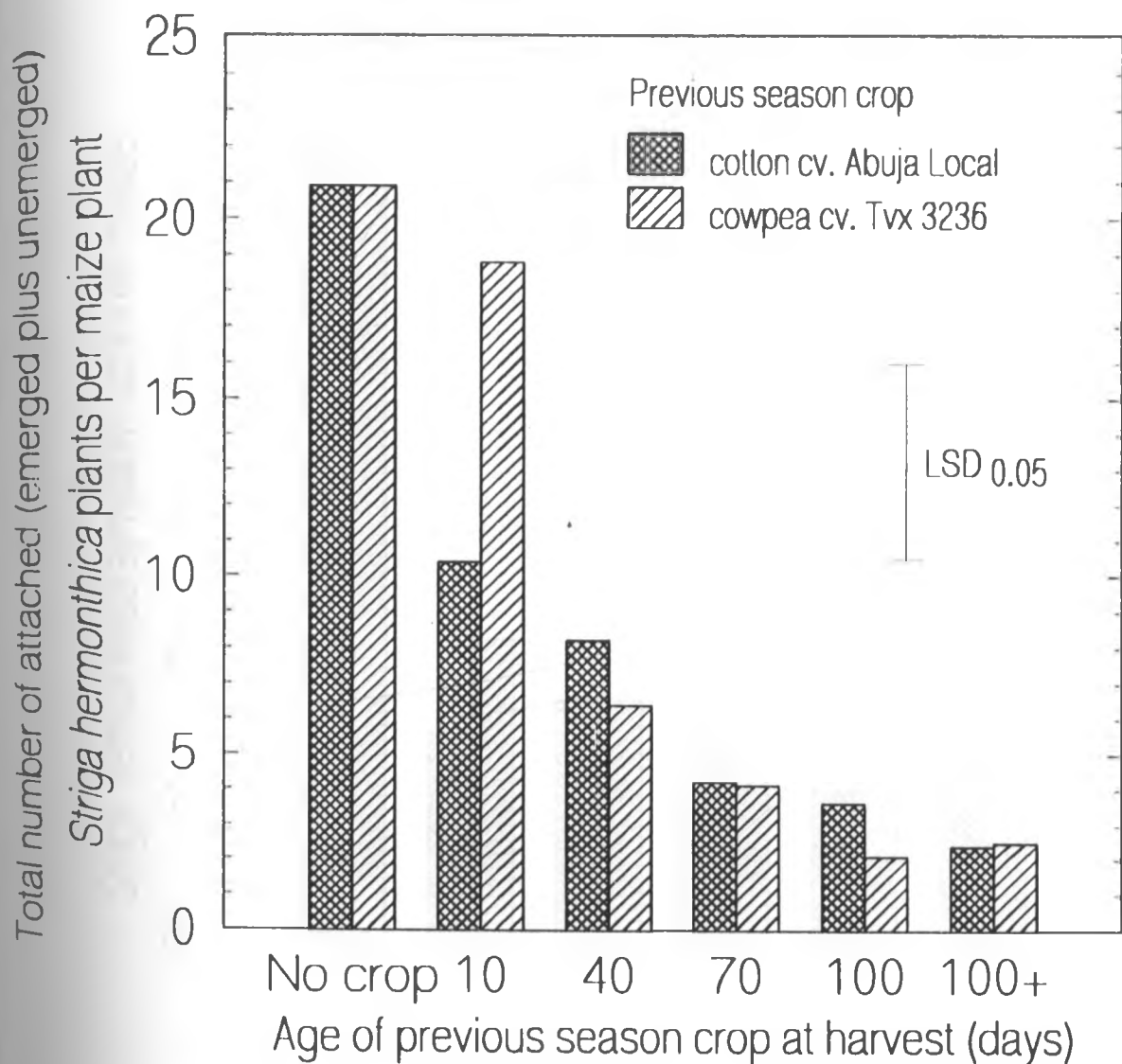
Fig. 7: Number of attached *Striga hermonthica* per maize plant.
A ' + ' denotes that 1 Kg or 2 Kg of fresh mulch from cowpea or cotton, respectively, was added, per ridge, before maize was planted.
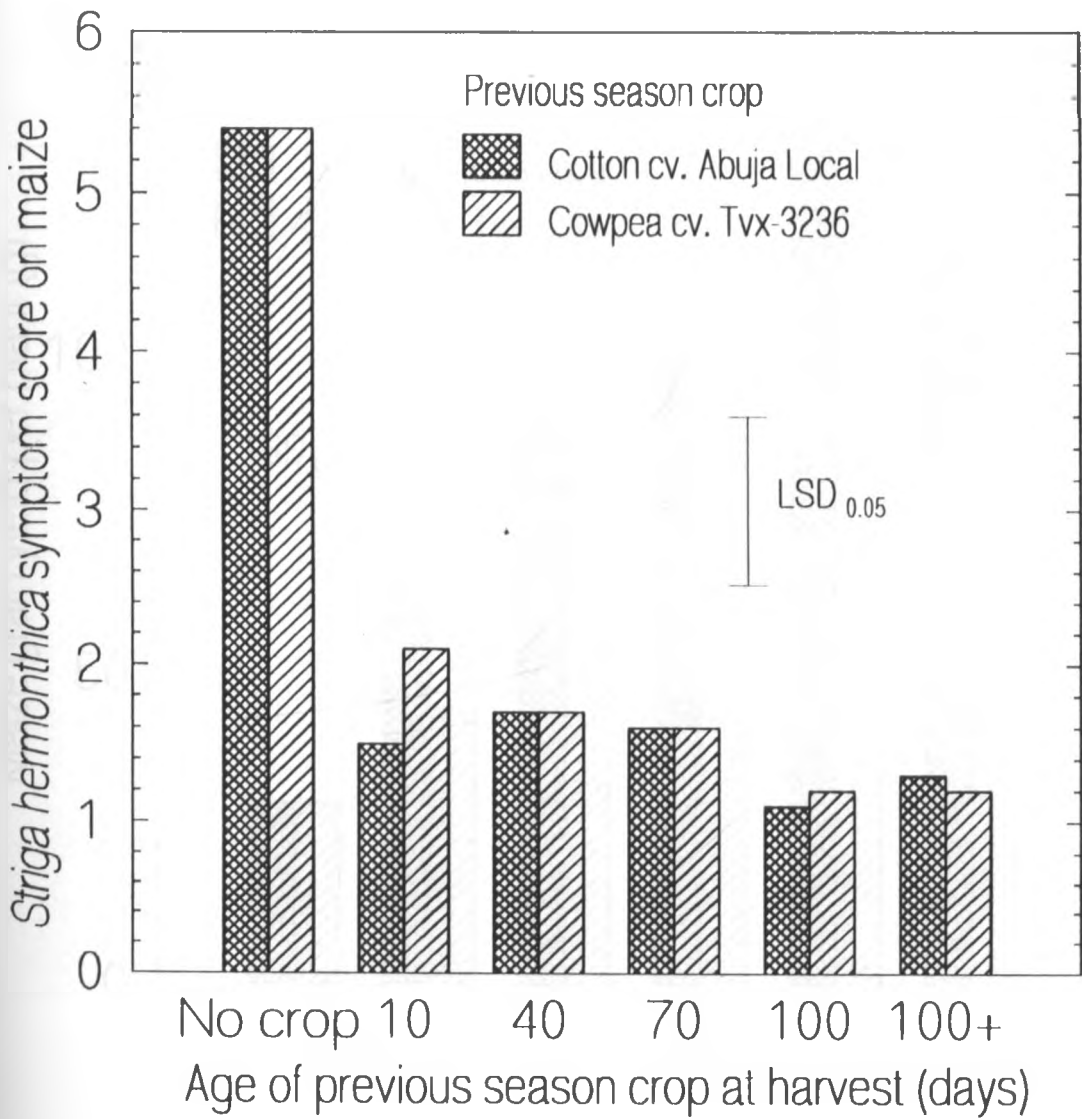
Fig. 8: Mean symptom score (1=No damage; 9=Heavy damage) on 10 week maize plants. A ' + ' denotes that 1 Kg or 2 Kg of fresh mulch from, cowpea or cotton respectively, was added, per ridge, before maize was planted.
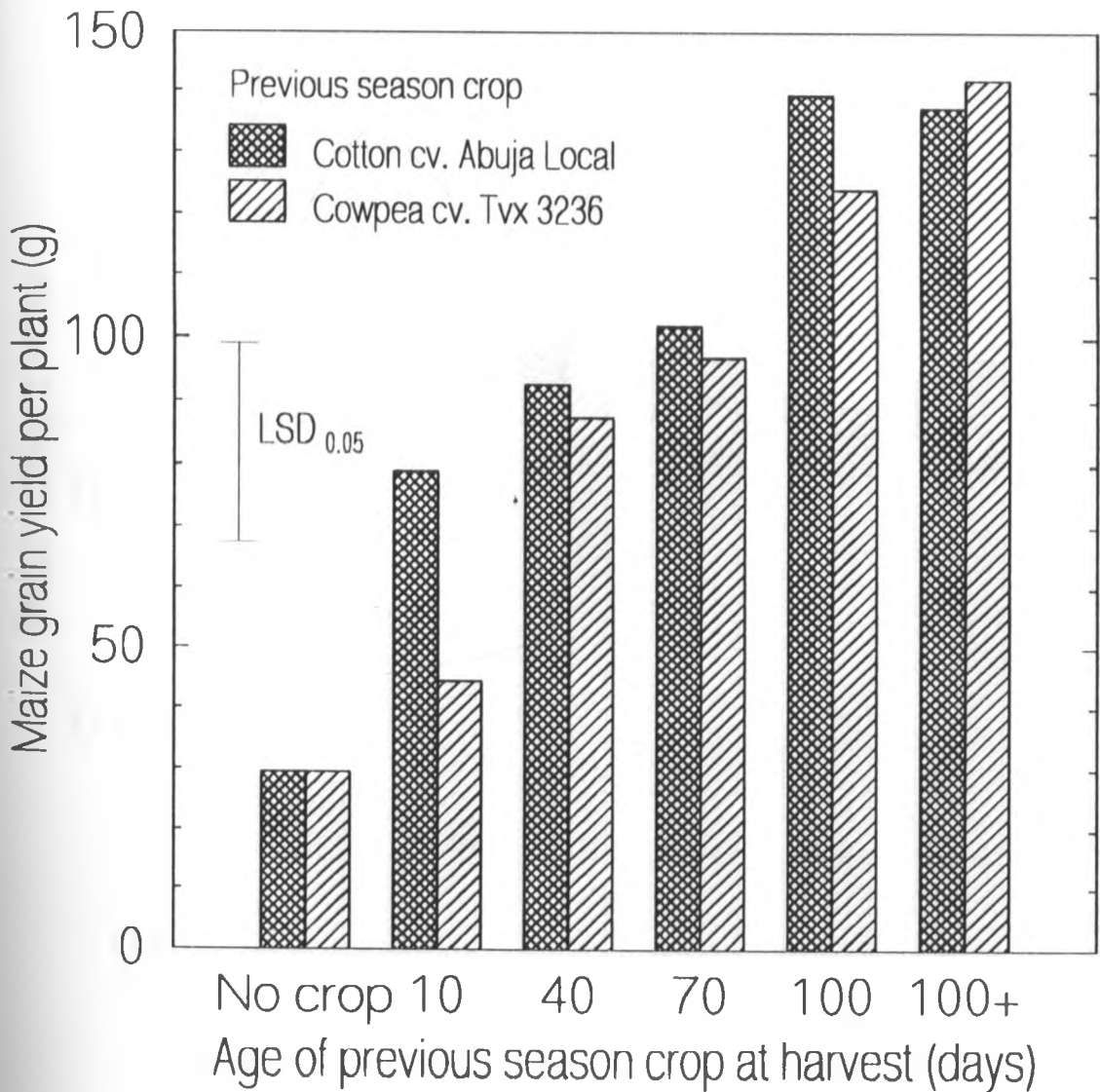
Fig. 9: Maize grain yield (g) per plant. 'A' denotes that 1 Kg or 2 Kg of fresh mulch from cowpea or cotton, respectively, was added, per ridge, before maize was planted.
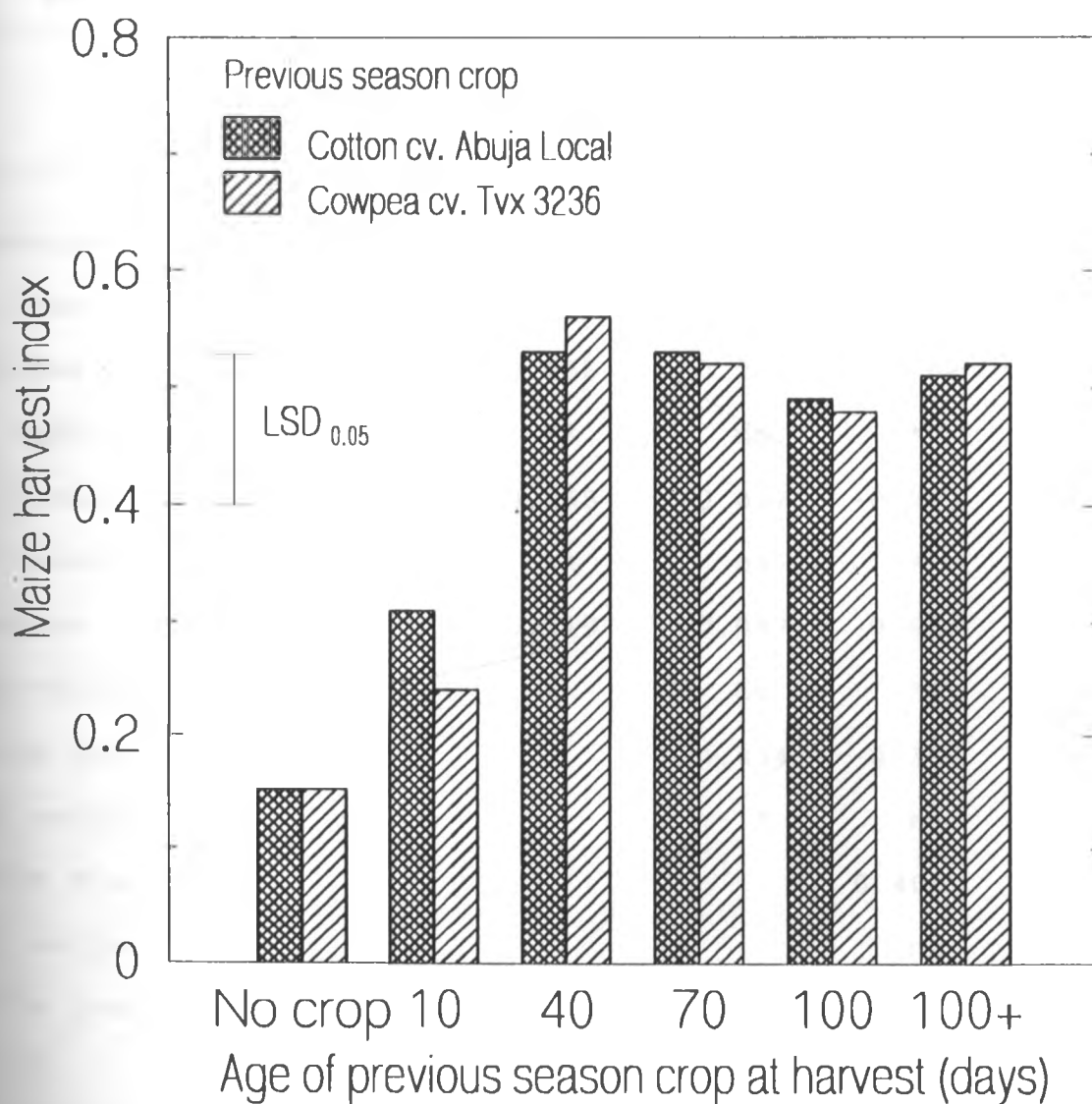
Fig. 10: Maize harvest index. A ' + ' denotes that 1 Kg or 2 Kg of fresh mulch from cowpea or cotton, respectively, was added per ridge, before maize was planted.

Number of unemerged, emerged, total attached *S. hermonthica* and root dry weight were positively correlated with symptom severity on maize (table 2). All parameters were negatively correlated with grain weight, and all, except stem dry weight, were negatively correlated with harvest index.

Table 2. Correlation coefficients (r) of *Striga hermonthica* parasitism and yield parameters of maize.

	Symptom expression	Root Weight	Stem Weight	Grain Weight	Harvest Index
Unemerged	0.599	0.540	-0.029	-0.689	-0.719
S. hermonthica	**	**	ns	**	**
Emerged	0.735	0.662	-0.032	-0.782	-0.835
S. hermonthica	**	**	ns	**	**
Attached	0.730	0.658	-0.032	-0.783	-0.834
S. hermonthica	**	**	ns	**	**
Symptom		0.319	-0.087	-0.638	-0.673
expression		*	ns	**	**
Maize root			0.454	-0.377	-0.728
dry weight			*	ns	**
Maize stem				0.408	-0.252
dry weight				ns	ns
Maize grain					0.754
yield					**

* and ** denote significant differences at P = 0.05 and P = 0.01, respectively, **ns** = not significant

2:4 DISCUSSION

2:4:1 Germination stimulation by dichloromethane soluble extracts.

In order to detect germination of *Striga hermonthica* seeds by dichloromethane soluble extracts from cotton and cowpea roots the extracts should be diluted to 0.5 and 1.0 percent DMSO respectively. However, for cotton leaves and cowpea stems the extracts should be diluted to 0.1 percent DMSO. Dimethlsulfoxide has been found to be less inhibitory than acetone (Pavlista *et al.*, 1979). Results indicated that extracts that stimulated germination of *Striga hermonthica* seeds have more than one identical compounds as shown by their Rfs. This indicates that perhaps more than one compound may be responsible for the parasite seed germination. The presence of more than one natural *Orobanche minor* stimulant(s) in faba roots and flax seed diffusate was reported (Khalaf *et al*, 1991).

Cowpea seed extract, although, showed very little germination activity with *Striga hermonthica* seeds also has closely related compound with other extracts. Perhaps the compounds in the seed have slight differences in their structures that render them inactive. *Striga hermonthica* seeds, as shown by the Rfs, had similar compounds to those found in other extracts. It should be noted that Brown (1946) and Brown and Edwards (1946) proved that

Striga asiatica seeds produced a germination stimulant during pretreatment. The first author hypothesized that the stimulant produced by the seed is similar to that supplied by the host. The amount produced however, is insufficient as exogenous stimulant is required. The spots represented by the low Rfs were so distinct that they could not have been left as impurities by advancing column of the crude extracts. However more work needs to be done to purify the extracts and perform *Striga hermonthica* seed bioassay at different Rf spots.

2:4:2 Effect of age of cotton and cowpea on germination stimulation

Aqueous extracts from cotton and cowpea plant parts, different growth stages of the crops contain germination stimulants for *S. hermonthica* seeds. Lack of stimulatory activity of aqueous extracts from cotton and cowpea seeds indicate that either *S. hermonthica* seed germination stimulant is not present in the seeds, but is synthesized after germination starts or that the stimulant may be present in the seeds inactive form which is activated by enzyme system after germination initiation. Saunders (1933) reported that liberation of the active germination stimulant of *S. asiatica* commenced as soon as root hairs formed on the developing radicle of maize. *S. hermonthica* seed germination stimulants is perhaps synthesized in the roots of cotton and cowpea seedlings, where it has the greatest activity, then translocated to the stem, leaves

and other parts of the plants. The diminishing germination activity of the extracts with increasing age of the crops suggests that perhaps the stimulants is used up by the growing cotton or cowpea or converted to inactive forms. At 70 days of growth the cowpea seeds were mature and the crop could be harvested to pave way for the next crop.

Both cotton and cowpea cultivars reduced *S. hermonthica* parasitism when grown prior to planting susceptible maize. The reason for this is putatively that the two non-host crops stimulated germination of parasite seeds in the absence of a host and reduced germinable parasite seed density in the soil. The reduction in number of *S. hermonthica* emergence on maize with increase in the period of cotton or cowpea growth the previous season indicate that the two crops continue to have a negative effect on *S. hermonthica* parasitism later in their growth cycle. Growing four plants of cotton for 10 days or one plant of cowpea for 40 days, reduced *S. hermonthica* parasitism on the following maize crop. This resulted in reduced symptom severity and higher grain yield and harvest index.

Number of emerged parasites and symptom severity increased with increase in maize root biomass. Since *S. hermonthica* germination stimulant is produced by maize roots, a larger root biomass may be associated with a higher stimulant production and greater number of attached parasites. Mulching was not additionally effective perhaps

because not enough time was allowed for the mulch leachate to induce parasite seed germination before maize was planted.

The short time period of 10 days growth required for cotton to reduce *S. hermonthica* parasitism on maize raises the possibility of planting cotton and plowing it under to stimulate parasite seed germination before planting a susceptible crop. More work needs to be done to determine the economic viability of this approach.

CHAPTER 3: EFFECTS OF COTTON, COWPEA AND SOYBEAN CROP RESIDUES ON *STRIGA HERMONTHICA* PARASITISM ON MAIZE.

ABSTRACT

Several researchers have found that exudates roots of host and non host plants, grown in water medium, stimulate germination of *Striga hermonthica* seeds. However, results indicate that cotton, cowpea and soybean residues also contain the parasite seed germination stimulants. Residues from cotton roots were more effective in stimulating the parasite seed germination than those from shoots at all distances and residue weights. Percent germination of *S. hermonthica* seeds induced by cotton, cowpea and soybean residues were dependent on the source of the stimulant, residue weight and the distance of the parasite seeds to the source of the stimulant.

Germination of the parasite seeds decreased with increase in residue weight. Compared to other residue weights, 25 g of cotton shoots and those from combined roots and shoots from soybean and cowpea significantly induced high percent germination of *S. hermonthica* seeds at all distances. Increasing residue weight for soybean and cowpea significantly reduced germination percent of *S. hermonthica* seeds at all distances. Germination of the parasite seeds by the two legumes increased with increasing distances from the source of the stimulant at all residue weights, except at 25 g for cowpea.

Germination of *S. hermonthica* seeds by cotton and cowpea plant parts were dependent on the source of the stimulant, plant part weight and the distance of the parasite seeds from the source of the stimulant. Germination of *S. hermonthica* seeds decreased with increase in plant part weight and period of time each cultivar was grown.

In separate experiments, effects of cowpea and soybean residues on *S. hermonthica* grown on maize was studied in potted soil in the screenhouse. At all incubation periods of cowpea and soybean residues increasing weight of residue delayed *S. hermonthica* emergence, reduced total number of parasites attached per maize plant and increased plant height and total dry matter yield of maize. At all residue levels, incubation period of the parasite seeds and the residues for at least 7 days was required to significantly reduce parasitism of *S. hermonthica* on maize.

3:1 INTRODUCTION

Germination of *S. hermonthica* depends on after ripening, conditioning in a warm moist environment for several days and subsequent exposure to an exogenous germination stimulant, usually produced as exudates from the roots of a number of host and non-host plants (Andrews, 1945; Mangnus *et al*, 1992; Smalling *et al*, 1991). Different host plants produce different mixtures of stimulants, as demonstrated by chromatographic investigation (Shaw *et al*, 1962).

Farm-yard manure (Watt, 1936) and compost (Timson, 1939) were reported to decrease the incidence of *Striga* species. It has been suggested that this decrease may be due to the induction of *Striga* species germination in the absence of its host, or to the adsorption of the host stimulant (Wilson-Jones, 1953), or possibly to the increase in soil moisture content or microbial activity of the soil.

Results from previous experiment indicate that aqueous extracts from roots and shoots of residues of non-host crops such as cotton (*Gossypium* spp.), cv. Abuja Local, and cowpea (*Vigna unguiculata* (L.) Walp., cv. Tvx 3236, induce germination of conditioned *S. hermonthica* seeds in the laboratory. These crops, which are part of farmers' cropping systems in Africa, can be included in rotations

to induce parasite seed germination and subsequent death in the absence of a host (Efron *et al*, 1989). To be adopted by African farmers, controls aimed at reducing *S. hermonthica* seed densities in the soil need to be inexpensive and allow land to be kept in production. The objective of this study was to determine the effects of incorporation of the residues of cotton, cowpea and soybean on parasitism of *S. hermonthica* on maize.

3:2 MATERIALS AND METHODS

3:2:1 Effective germination distance of S. hermonthica seeds by aqueous extracts from crop residues

Cotton, cowpea and soybean cultivars (Abuja Local, Tvx-3236, and TGX-1674-1F) respectively, were grown upto maturity, in Abuja (9.12 N, 7.20 E) in Nigeria. They were harvested and their roots separated from shoots and washed. The samples were dried at 60°C for 3 days (Chidley and Drennan, 1987). The samples were ground to pass through a 2 mm sieve and used in this experiment.

Two (Whatman No.1) filter papers were placed in a 9.0 cm petri dish and moistened with 3 ml distilled water to provide partial seal. Discs containing the parasite seeds were removed from conditioning petri dishes dabbed on dry filter paper to remove excess moisture. Four rows of four discs containing the conditioned *S. hermonthica* seeds were arranged perpendicularly to a 20 mm ring, of aluminum foil, placed at the center of the petri dish. The dry ground samples of cotton, cowpeas and soybean weighing 0.25, 0.50, 0.75 and 1.0 g were placed at the center of the ring and 3.0 ml of distilled water was applied using micro-pipette. The petri-dishes were sealed with paraffin film and incubated in the dark at 30°C for 48 hours. Germinated *S. hermonthica* seeds were counted under the microscope X20 magnification. The experiment was replicated four times and repeated twice and average germination percent was

determined. Analysis of variance was carried to test differences between means.

3:2:2 Incubation of crop residues and parasite seed in the soil.

Pot of 20 cm in diameter were filled with clean top soil. The top 15 cm of the soil in each pot, except the control, was removed and mixed with the dry, ground cotton, cowpea and soybean crop residues at 0, 2.0, 4.0 for cotton and an additional 8.0 and 16 g per pot for cowpea and soybean respectively. A mixture of soil, 1000 germinable *S. hermonthica* seeds, and the residues was returned in each pot. The pots were arranged in a complete randomized design and replicated four times. The pots were watered by adding 400 ml of water every 2 days and incubated for 28, 21, 14, 7, or 0 days before planting. Three seeds of *S. hermonthica* susceptible maize variety, 8338-1, were planted in each pot and thinned to one 7 days later.

Treatments without crop residues but infested with *S. hermonthica* seeds were used as a control. These were also replicated four times. Day of first *S. hermonthica* emergence and the number of emerged parasite plants were recorded every week until harvesting at 120 days after planting. Maize plant height was taken at maturity. At harvesting, each pot was emersed in a series of buckets of water to remove the soil and the underground attached *S. hermonthica* plants were counted. The roots were separated from

the shoots, placed in paper bags, labelled and dried at 60°C for 3 days, after which their dry matter yields were recorded. Data was subjected to analysis of variance.

3:3 RESULTS

3:3:1 Germination of *S. hermonthica* seeds caused by crop residues

Results (figures 1 and 2) show that residues from cotton roots were more effective in stimulating the parasite seed germination than those from shoots at all distances and residue weights. Percent germination of *S. hermonthica* seeds was dependent on the source of the stimulant, residue weight and the distance of the parasite seeds to the source of the stimulant.

Cotton shoot residues induced lower germination percent of *S. hermonthica* seeds than the root at all levels of residue weight and distance of the parasite seeds from the source of the stimulant (table 1). Whereas the effect of cotton root residue was significant, that of the shoot was not significant. There was no significant interaction between residue weight and distance for cotton residue but this was significant for the shoot at $P=0.05$. There was an increase in germination of the parasite seeds with increase with distance to a maximum, at 16 mm from the source of the stimulant, then declined with further increase in distance upto 32 mm.

Fig. 1: Germination of *S. hermonthica* seeds in response to distance of the parasite seeds from varyng weights of cotton root residues

Fig. 2: Germination of *S. hermonthica* seeds in response to distance of the parasite seeds from varyng weights of cotton shoot residues

Table 1: Mean percent germination of *S. hermonthica* seeds as affected by residue weight and distance of the parasite seeds from the source of the stimulant.

Residue weight (g)	Germination percent	
	Cotton root	cotton shoot
0.25	68.4ª$	33.2ª
0.50	66.0ª	36.4ª
0.75	54.4ᵇ	35.5ª
1.00	61.5ᵃᵇ	36.2ª
GR24#	59.5ᵃᵇ	31.9ª
Distance (mm)		
8	69.7ª	42.8ª
16	71.1ª	29.3ᵇ
24	58.9ᵇ	24.9ᵇ
32	48.1ᶜ	41.7ª
Significance		
Residue weight	*	NS
Distance	***	***
Weight X Distance	NS	*

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability respectively.

GR 24 at a concentration of 10^{-2} mg L^{-1} was included as a control.

$ Within columns, for each factor (residue weight or distance), means followed by the same letter are not significantly different at the 0.05 level, according to ANOVA-protected Duncan's Multiple Range Test.

Residues of both soybean and cowpea cultivars, at 0.25 g, induced significantly high percent germination of *S. hermonthica* seeds at all distances. However maximum germination of the parasite seeds was obtained at 32 and 24 mm for soybean and cowpea residues respectively after which germination decreased with further increase in distance. Increasing residue weight for soybean (figure 3) and cowpea (figure 4) significantly reduced germination percent of *S. hermonthica* seeds at all distances. Germination of the parasite seeds increased with increasing distances from the source of the stimulant.

3:3:2 Effects of incubation of the parasite seed with crop residues

At all incubation levels, increasing residue weight of cowpea significantly increased the first date of the parasite emergence (figure 5). Compared to other treatments maize plant height and total dry matter yield were significantly lower when the parasite seeds were incubated with different levels of cowpea residues for 28 days before maize was planted. Incubation period of the residues with the parasite seeds had little effect on total *Striga*
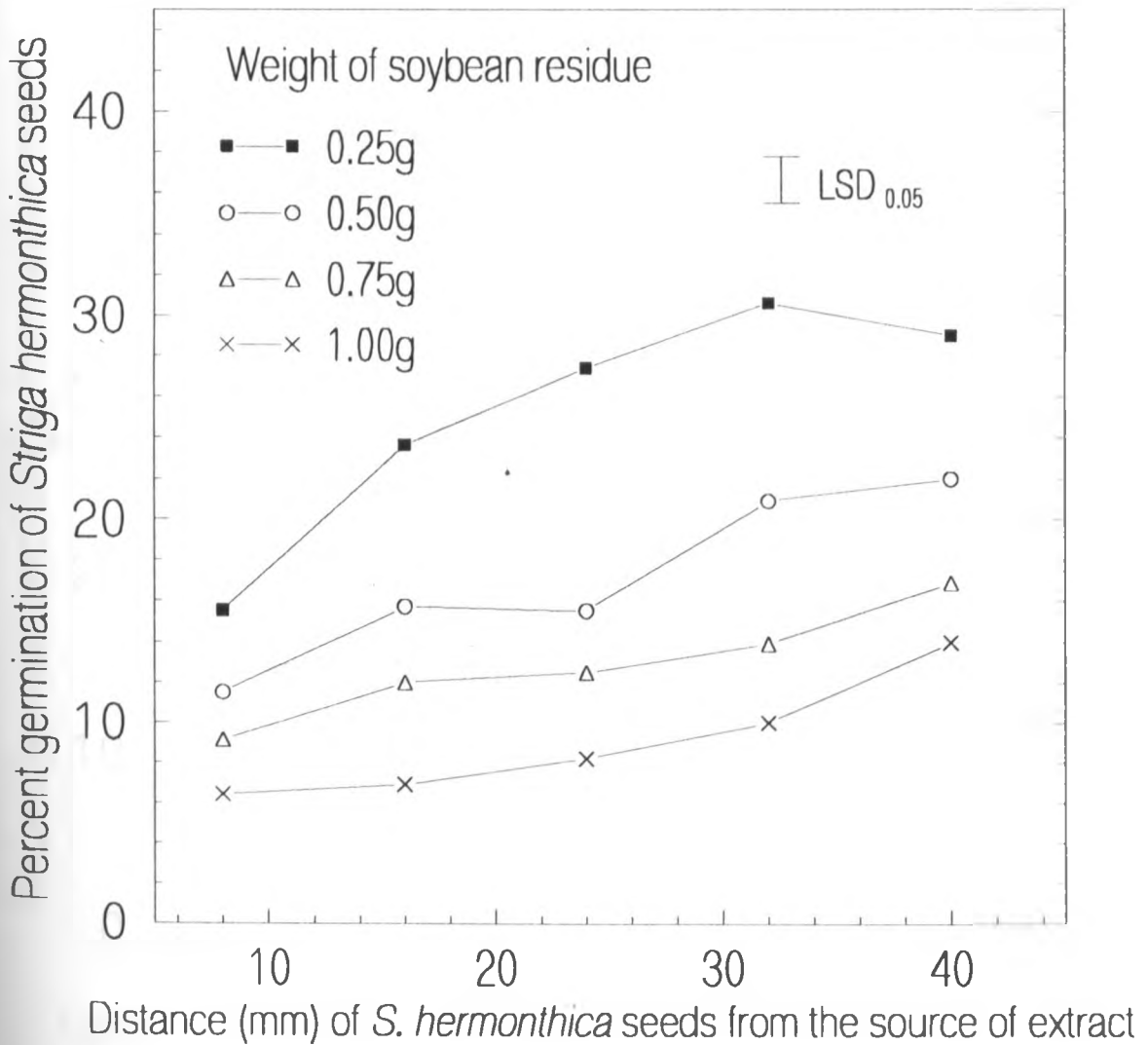
Fig. 3: Percent germination of *S. hermonthica* seeds in response to distance of the parasite seeds from varyng weights of dry root and shoot residues of soybean cultivar, Samsoy 2.

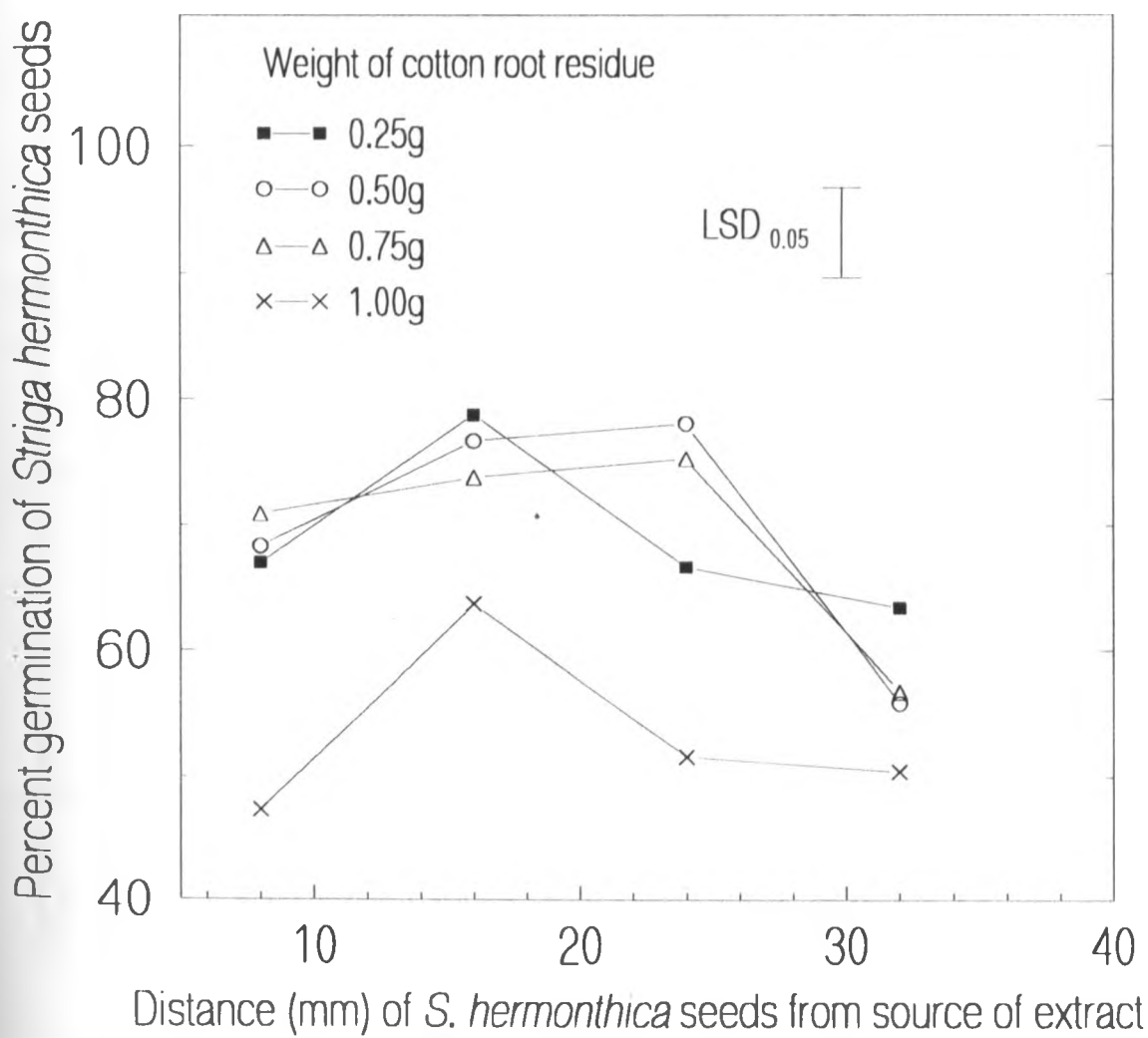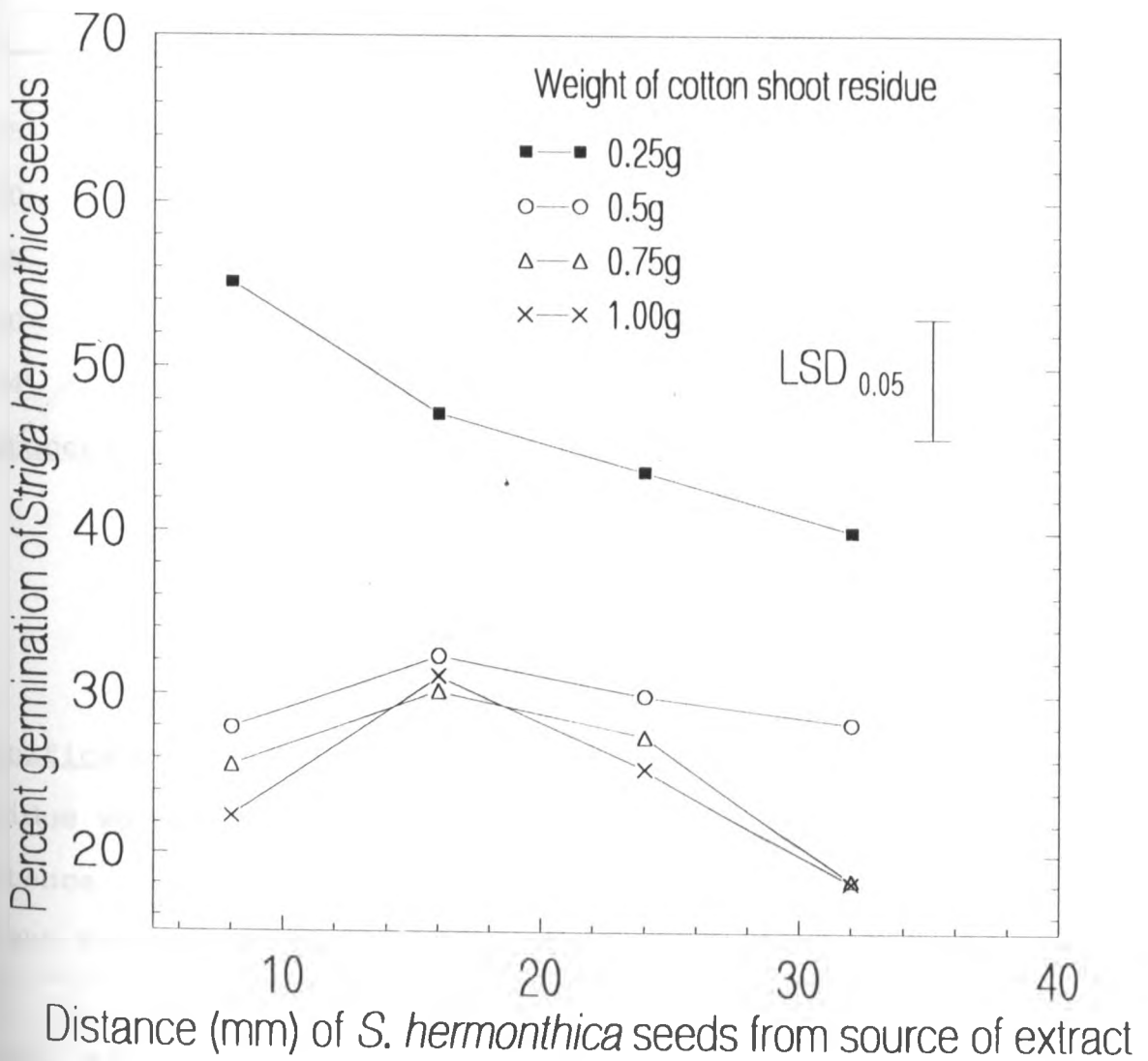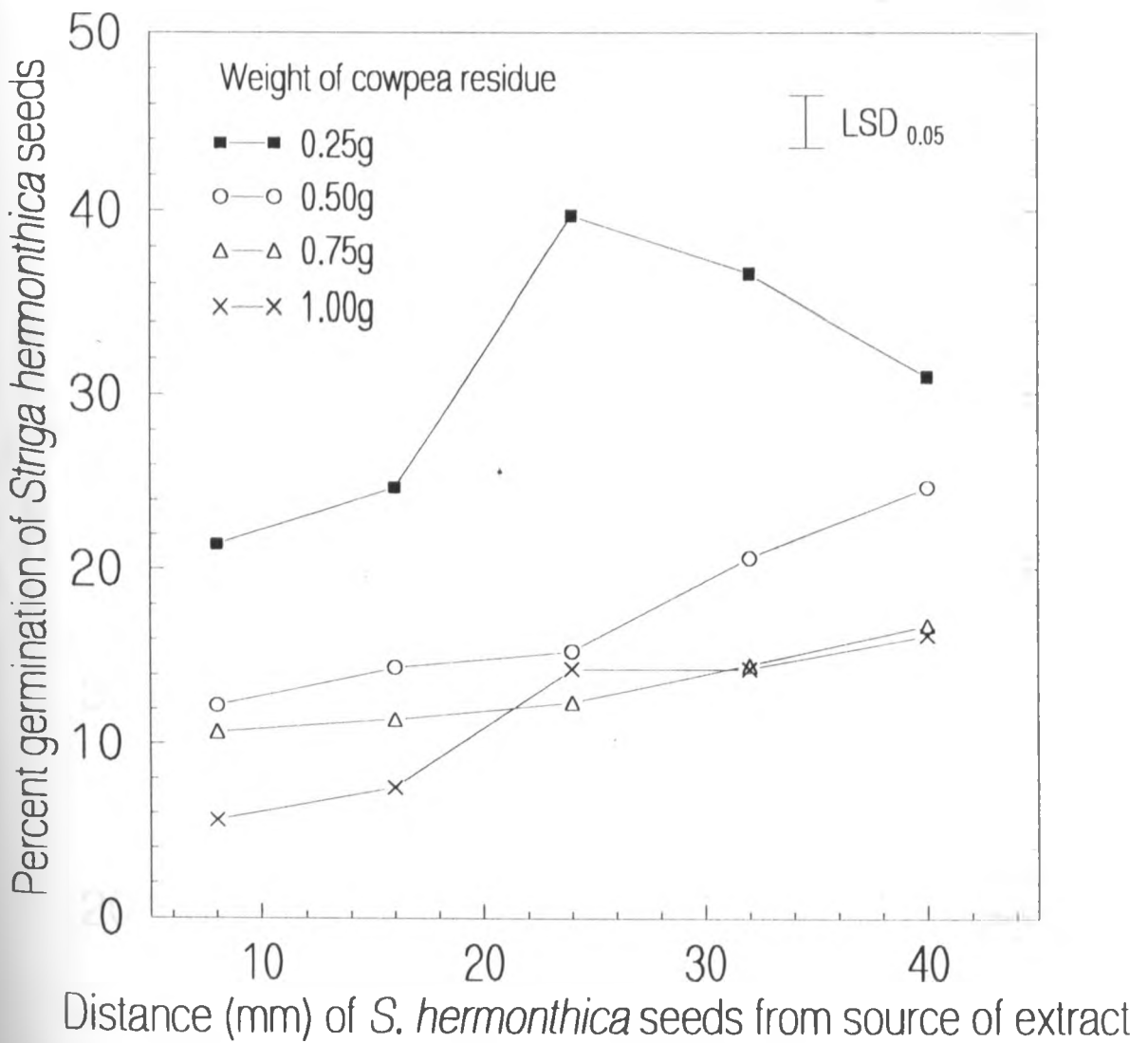Fig. 4: Percent germination of *Striga hermonthica* seeds in response to distance of the parasite seeds from varyng weights of dry root and shoot residues of cowpea cultivar, Tvx-3236.
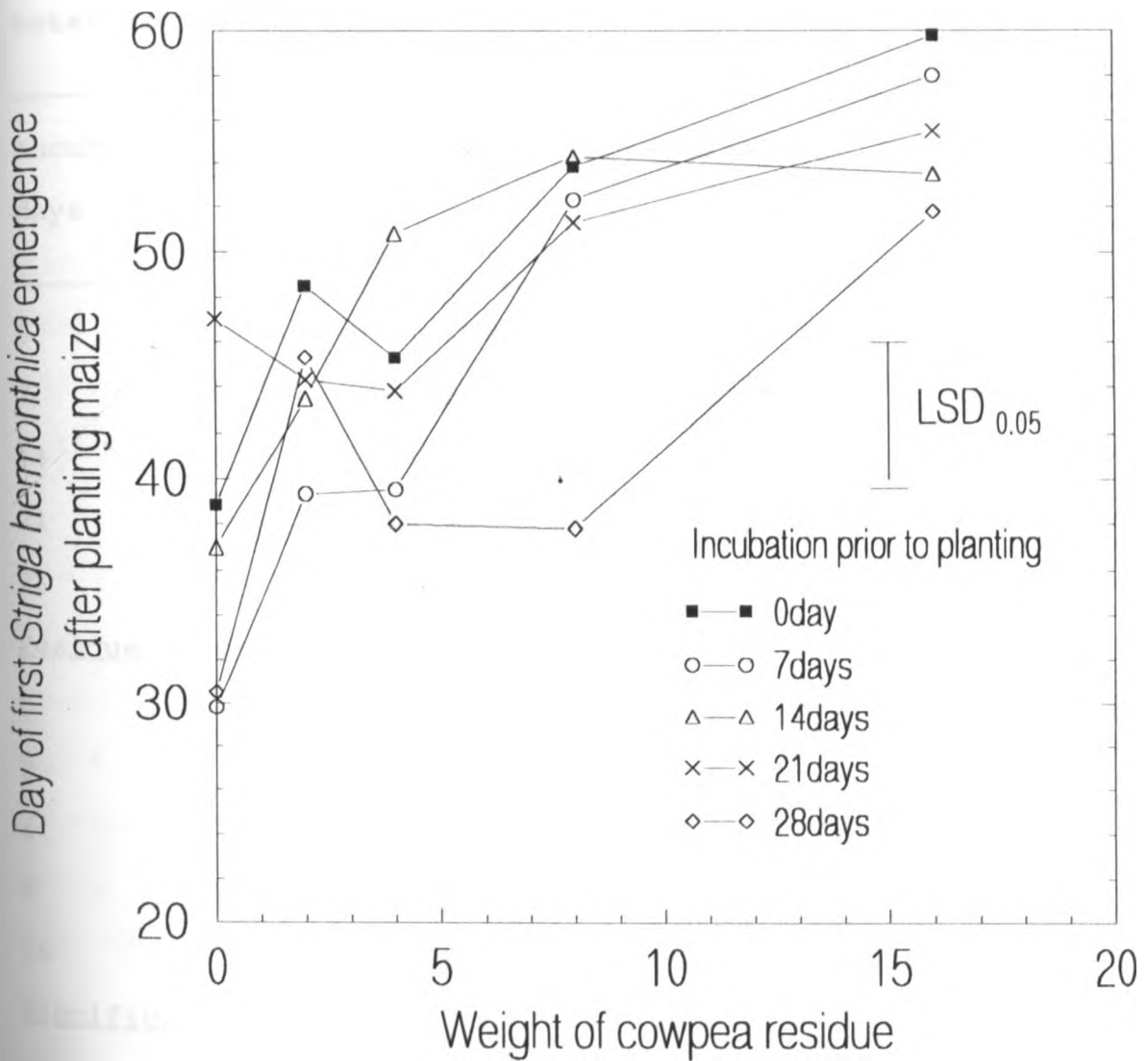
Fig. 5: Day of first *S. hermonthica* emergence after planting maize in pots infested with the parasite seeds and incubated with root and shoot residues from cowpea cultivar, Tvx 3236, for varying number of days.

hermonthica plants attached to maize root at harvest. However, the number of attached parasites decreased with increasing residue weight and incubation period (table 2).

Table 2: Effects of residue weight of cowpea and its incubation period on total *S. hermonthica* attached, maize plant height and total dry matter yield.

Incubation days	Total *Striga*	Maize HT (cm)	Maize DM (g)
0	14.6^{ab}\$	147.0^a	44.4^a
7	12.2^{ab}	135.2^{ab}	41.3^a
14	11.2^{ab}	154.5^a	43.0^a
21	14.9^a	148.8^a	41.6^a
28	10.2^b	119.8^b	34.3^b
Residue weight (g)			
0	14.7^a	107.3^d	37.2^c
2	16.4^a	126.9^{cd}	38.6^c
4	15.1^a	144.2^{bc}	38.6^c
8	10.1^b	159.2^{ab}	42.8^b
16	6.6^b	166.8^a	47.3^a
Significance			
Days	NS	**	***
Weight	***	***	***
Days X Weight	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability respectively.

$ Within columns, for each factor (incubation period or residue weight), means followed by the same letter are not significantly different at the 0.05 level, according to ANOVA-protected Duncan's Multiple Range Test.

HT, height; **DM**, dry matter; **NS**, not significant.

At all incubation levels, increasing cowpea residue weight decreased total number of *Striga hermonthica* attached on each maize plant. Cowpea residues of 16 grammes followed by 8 grammes were significantly effective in reducing the number of the parasites attached on each maize plant and increasing total dry matter yield of the crop.

Date of first *S. hermonthica* emergence increased with increasing amounts of soybean residues, reaching maximum at 4, 8 and 16 grammes of residues when incubation periods were 7, 14 and 0 and 21 days respectively (figure 6). Total number of *S. hermonthica* plants attached on each maize plant was significantly higher, at all levels of incubation period, when no soybean residue was incubated with the parasite seeds before maize was planted. Addition of any level of soybean residue significantly increased maize plant height and total dry matter yield compared to the treatments without residue (table 3).
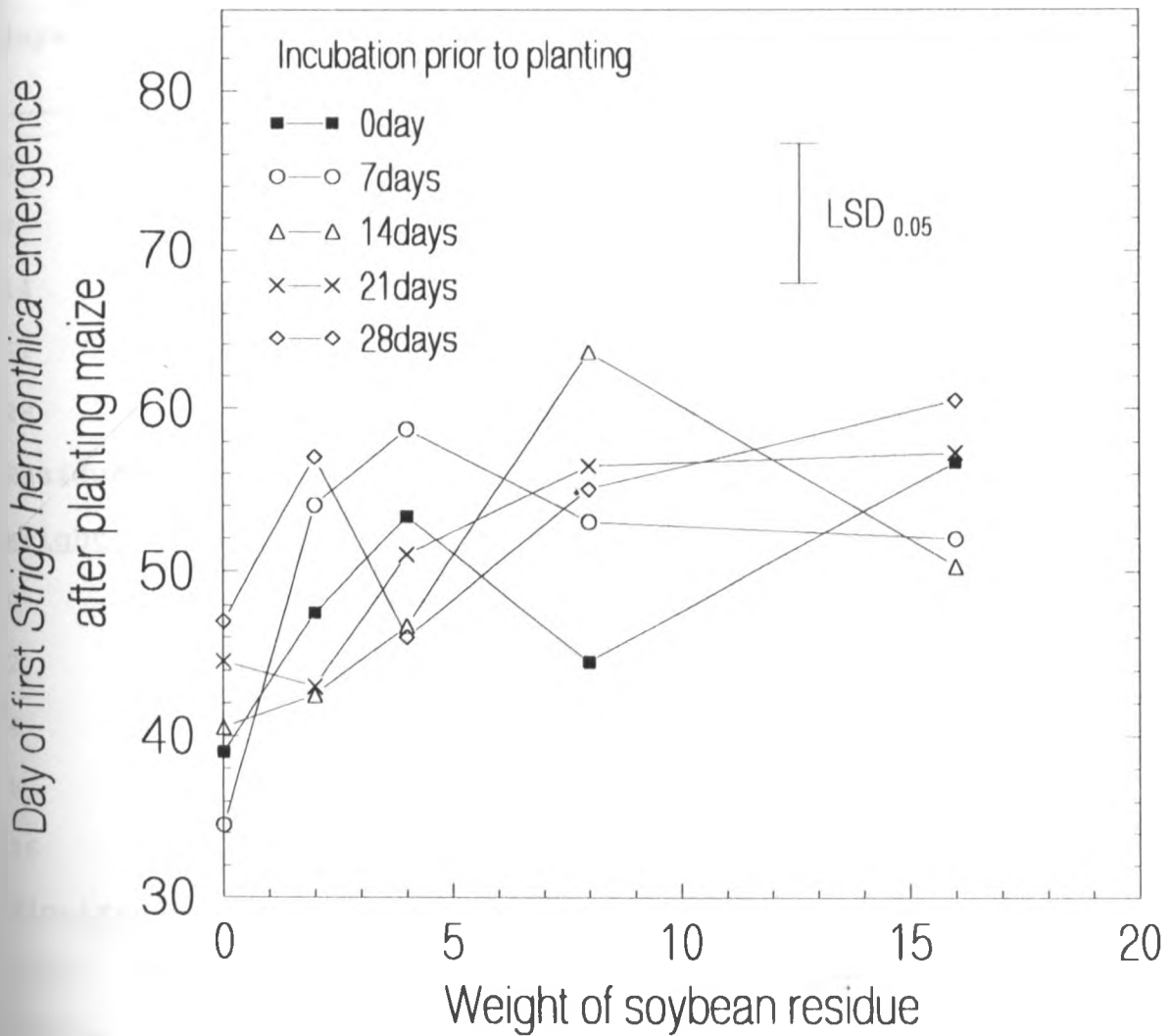
Fig. 6: Day of first *S. hermonthica* emergence after planting maize in pots infested with the parasite seeds and incubated with root and shoot residues from soybean cultivar, Samsoy 2, for varying number of days.

Table 3: Effects of residue weight of soybean its incubation period on total *S. hermonthica* attached, maize plant height and total dry matter yield.

Incubation days	Total *Striga*	Maize HT (cm)	Maize DM (g)
0	8.9^a$	130.4^b	15.2^c
7	4.8^{ab}	160.2^a	22.5^{ab}
14	4.1^b	159.3^a	17.1^{bc}
21	5.9^{ab}	170.8^a	19.7^{abc}
28	6.1^{ab}	173.6^a	23.7^a
Residue weight (g)			
0	9.8^a	150.5^a	18.5^a
2	5.4^b	159.8^a	18.3^a
4	5.3^b	151.6^a	17.0^a
8	3.8^b	167.1^a	22.0^a
16	5.4^b	165.3^a	22.3^a
Significance			
Days	NS	***	**
Weight	*	NS	NS
Days X Weight	NS	NS	NS

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability respectively.

\$ Within columns, for each factor (incubation period or residue weight), means followed by the same letter are not significantly different at the 0.05 level, according to ANOVA-protected Duncan's Multiple Range Test.

HT, height; **DM**, dry matter; **NS**, not significant.

3:4 DISCUSSION

3:4:1 Germination of *S. hermonthica* seeds caused by crop residues

The high level of germination percent of *Striga hermonthica* seeds induced by aqueous diffusate from root and shoot residues of cotton (Abuja Local), cowpea (Tvx-3236) and soybean (Samsoy-2) indicate that harvesting the lint and seeds of these crops and plowing the crop residue under in infested fields would cause the parasite seeds to germinate and die in the absence of a host. This would deplete the parasite seed reservoir in the soil. The low residue weight required to achieve maximum *S. hermonthica* seed germination would enable incorporation of a small quantity of cotton, cowpea and soybean residues that would not detrimentally upset the C/N ratio of the soil thereby affecting yield of the following season crop.

Germination of the parasite seeds upto 32 mm away from cotton and 40 mm from cowpea and soybean residues would increase the chances of stimulating germination of the parasite seeds away from the residues. Depression of *S. hermonthica* seed germination by residues from cotton, cowpea and soybean residues as the weight of the residues increase suggests the presence of germination inhibitors. Residues from cotton shoots depressed germination of the parasite seeds more than those from roots. This indicates, perhaps, that shoots contain more germination inhibitors than

3:4:2 Effects of incubation of the parasite seed with crop residues

Addition of cowpea and soybean residues to the soil after harvest is beneficial in reducing *S. hermonthica* parasitism on maize the following season. Incorporation of cowpea or soybean residues for at least 7 days is necessary to achieve the best result. Rainfall during the period of incubation is necessary to allow for leaching of the parasite germination stimulant from the residues. The parasite seeds which are germinated would, within the 7 days of incubation, die in the absence of a suitable host. Without allowing time for incubation, the parasite seeds are stimulated to germinate by aqueous extracts from cowpea or soybean residues and then attaches themselves to maize plant. This explains why *S. hermonthica* emerged early, in large numbers and reduced plant height and total dry matter yield of maize in the treatments where incubation time was 0 day.

The reduction of the *S. hermonthica* parasitism on maize after residues of the cowpea and soybean were incubated with the parasite seeds cannot be attributed to nitrogen availability as it has been reported that mineral fertilizers including nitrogen and farmyard manure did not significantly reduce *S. hermonthica* infestation on maize (Smalling *et al*, 1991 and Osman *et al*, 1991). Nitrogen seems to neutralize the harmful effects of the parasite without reducing the extend of parasitism (Osman *et al*, 1991) .

CHAPTER 4: EFFECTS OF COTTON, COWPEA , SOYBEAN AND THEIR RESIDUES ON FIELD MANAGEMENT OF *STRIGA HERMONTHICA*

ABSTRACT

Striga hermonthica (Del.) Benth. is a severe parasite on maize and a limiting factor in achieving optimum maize yields in infested areas in Africa. The parasite seeds are stimulated to germinate by root exudates from host and non-host plants as well as by synthetic germination stimulants. Field effectiveness of cowpea (Tvx 3236, IT-87D-1951), soybean (Samsoy-2, TGX-1674-1F) and cotton (Abuja Local) rotations and use of their residues to control *S. hermonthica* in maize were evaluated. All rotations significantly reduced the number of attached parasites on maize plants and increased grain yields the following season. Addition of residues from Tvx 3236 significantly reduced parasitism of *S. hermonthica* on maize and increased yields.

4:1 INTRODUCTION

Striga hermonthica (Del.) Benth. is an extremely damaging parasite in maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), millet (*Pennisetum glaucum* (L.) R. Br.), sugarcane (*Saccharum Officinarum* L.) and upland rice (*Oryza sativa* (L.) in most regions of Africa (Saunders, 1933; Shaw *et al*, 1962; Doggett, 1965; Parker *et al*, 1971), where witchweed causes yield losses ranging from 10 to 100 percent (Ramaiah, 1983). A single *Striga hermonthica* plant is able to produce up to 500,000 seeds which mature at different times (Andrews, 1947). The seeds do not all germinate at the same time, but continue to remain viable in the soil for up to 20 years (Saunders, 1933; Vasudeva Rao *et al*, 1989). The long viability in combination with high seed production result in severely infested fields.

Although parasite seeds usually respond to host plant exudates, some non-host plants also produce exudates which will germinate the parasite seeds (Andrews, 1947; Brown, 1965; Sahai and Shivana, 1982; Worsham, 1987). *Striga hermonthica* parasitizes a number of species but soybean, lucerne (*Medicago sativa* L.) and sun-hemp (*Crotalaria juncea* L.) and dolichos bean (*Dolichos lablab* L.) (Andrews, 1947) and cotton (Doggett, 1953) are suitable trap crops. Other trap crops documented include pigeon pea (*Cajanus cajan* L.), green gram (*Phaseolus aureus* L.), black gram (*Phaseolus mungo* L.) and sesamum (*Sesamun indicum L.*) (Andrews, 1947 and Hosmani, 1978).

The authors noted that further documentation of the efficacy of the trap crops in reducing soil population of *Striga* spp. is needed. Differences in varietal reaction should not be forgotten (Andrews, 1947). In Kenya, it was noted that growing of cassava crop for three years almost freed the land of *S. hermonthica* (Watt, 1936).

Several workers recommend the use of trap crops in the rotation to deplete the reservoir of witchweed seed in the soil (Andrews, 1947; Doggett, 1970; Ramaiah and Parker, 1982; and Wilson-Jones, 1953). Parkinson *et al*, (1988) showed that the *S. hermonthica* population in the plot under three years continuous cropping with soybean was significantly lower than the plot under continuous maize for a similar period of time. Bambara nut was found to be another suitable trap crop in the drier Sudan and Sahel savannas where neither cotton nor soybean can be grown as trap crops. In another experiment, to study the induction of *S. hermonthica* germination root exudates, Bebawi and Michael, (1991) reported that hyacinth bean (*Dolichos lablab* L.) had the highest stimulation followed by cotton (*Gossypium barbadense* L.), guar [*Cyamopsis tetragonoloba* (L.) Taub.], sesame (*Sesamum indicum* L.) and sunflower (*Helianthus annus* L.). These trap crops, which are part of farmers' cropping systems in Africa, can be included in rotations to induce parasite seed germination and subsequent death in the absence of a host (Eplee, 1975). To be adopted by African farmers, controls aimed at reducing *S. hermonthica* seed densities in the soil need to be inexpensive and allow land to be kept in production. The

objective of this study was to determine the effects of the previous season crop of cotton, cowpea and soybean and application of their residues on parasitism of *S. hermonthica* on maize the following season.

4:2 MATERIALS AND METHODS

4:2:1 Effects of crop rotation on *Striga hermonthica* on maize

The experiment was done under rain-fed condition in Abuja, (9.12N, 7.20E) in Nigeria, which is in a *Striga hermonthica* infested area. A total area of 30 m by 100 m was used. Land was ploughed and harrowed before marking the plots. A Complete Randomized Block Design was used, with the treatments replicated five times. Each plot measured 8.5 m X 5.0 m, giving 12 rows of 75 meters apart.

Planting was done at the onset of rains with cowpea (IT-87D-1951 and TVX-3236), soybean (Samsoy 2 and TGX-1674-1F), cotton (Abuja Local) and a susceptible maize variety (8338-1), giving a total of thirty plots. Alleys measuring 4 m were left between tiers, with one tier having 36 rows which are 5.0 m long. Top and sub-soil samples were taken from the field before planting, using an Auger. The samples were taken to analytical laboratory for physical and chemical analysis. These included percent clay, silt and sand, amount of organic carbon, phosphorous, potassium, calcium, and magnesium. Other analysis included pH and cation ion exchange capacity.

All rows, excluding alleys, were infested with 3.33 g of *S. hermonthica* per row, containing 186,480 germinable seeds. The *S. seeds* used were collected from sorghum in Abuja in 1991. The *S.*

hermonthica seed was mixed with sand in proportion to give a 1.0% concentration of *Striga* seed w/w before infesting in the furrow. The plots and rows were permanently marked for planting the following season. Maize was planted at a spacing of 75 cm by 25 cm with one seed per hole. A compound fertilizer formulation of 15-15-15 was applied at the rate of 40 Kg N/ha at the time of planting.

The spacing of cowpea was 75 cm by 25 cm, with 2 seeds/hole, while soybean was drilled 4 cm apart and 75 cm between the rows. Cotton was planted at a spacing of 75 cm by 25 cm and received the same amount of a compound fertilizer. All weeds were removed by hand hoeing and pests and diseases were controlled. Number of *S. hermonthica* plants emerged in each plot was recorded fortnightly. At harvesting, each plot measuring 12 rows (75 cm) by 5 m was divided into two plots measuring 6 rows (75 cm) by 5 m. The trap crops were harvested and their seeds removed. The crop residue were weighed and spread above the soil in half the plots. In the remaining half, the residues were removed.

During the next season, (1994) susceptible maize variety (8338-1) was planted in all the plots, with minimum disturbance of the soil. All weeds were removed. The date of first *S. hermonthica* emergence was recorded in each row. The parasite count was taken weekly until harvesting. Visual damage was scored using symptomatic expression to assess the *S. hermonthica* damage. A scale of 1-9 was used, where 1 = No damage and 9 = Heavy damage (Kim, 1991). Other data taken

included cob and plant height (cm), number of cobs per plant and grain yield (grames per plot). Statistical analyses was used to compare treatments to the control. A significance level of 0.05% was used.

4:3 RESULTS

4:3:1 Soil physical and chemical analysis

Results of soil physical and chemical analysis are shown (table 1).
The soils are acidic and deficient in nitrogen, phosphorous
and potassium.

**Table 1: Physical and chemical properties of soils at IITA, Abuja,
   outreach research station where field experiments were carried
   out in 1993 and 1994.**

Soil properties	Values
pH (H_2O)	5.72
Organic Carbon (%)	0.42
Total Nitrogen (%)	0.05
Available phosphorous (mgP/g)	4.44
Calcium (meq Ca/100g)	1.15
Magnesium (meq Mn/100g)	0.18
Manganese (meq Mn/100g)	0.05
Potassium (meq K/100g)	0.17
Sodium (meq Na/100g)	0.61
Total acidity (meq/100g)	0.16
Cation Exchange Capacity (meq/100g)	2.31
Texture	Loamy Sand

The level of organic matter is low which contributes to poor physical properties of the soil. The soil is easily erodible under high rainfall intensity.

4:3:2 Climate at Abuja (1993 / 1194)

(i) Air and Soil Temperatures and Relative Humidity

The lowest minimum air temperature was recorded in December 1993 and January, 1994 (appendix 1). This coincided with the Hamattan, a period of low air temperature, humidity and rainfall. The trend was also true for soil temperatures at 10 and 20 cm depths respectively. Higher air and soil temperatures were recorded in March, 1994. Lowest relative humidity was recorded from October 1993 to December 1994 and was high between April and August 1994 (appendix 2). Soil temperatures at 10 and 20 cm respectively had the same pattern throughout the two years (appendices 3 and 4). However wide variations between minimum and maximum were recorded at 10 compared to 20 cm depth.

(ii) Rainfall and Photosynthetic Active Radiation

There was a prolonged raifall starting from April to October during both years (appendix 5). Peak rainfall was received in September 1993 and August 1994 respectively. Planting cowpea, soybean, cotton

and maize was done in June 1993. This was done to coincide with harvesting during low rainfall period in October for cowpea, soybean and maize and in December for cotton. Maize was planted in June 1994 and harvested in October the same year. December to March was dry. Photosynthetic active radiation was high during the two growing seasons (appendix 6). This was beneficial to crops under the non-limiting moisture conditions except between December to March when moisture was limiting. The climate was conducive to crop growth during the two years.

4:3:3 Effects of crop rotation on *S. hermonthica* on maize

Cotton (Abuja Local), soybean (TGX-1674-1F and Samsoy-2) and cowpea (IT-87D-1951) were erect while cowpea (Tvx-3236) was creeping. All the crops were equally vigorous with good ground cover. Grain yields of the two soybean and cowpea cultivars and the two legumes were not significantly different during 1993 long rainy season (figure 1).

During 1994 season, no significant differences were observed in maize height and number of cobs per maize plant in plots planted to cowpea, soybean, cotton or maize during 1993 season. Growing the two cowpea cultivars and application of their residues after harvest in 1993 season before planting maize the following season (1994) significantly increased cob height of maize (figure 2).
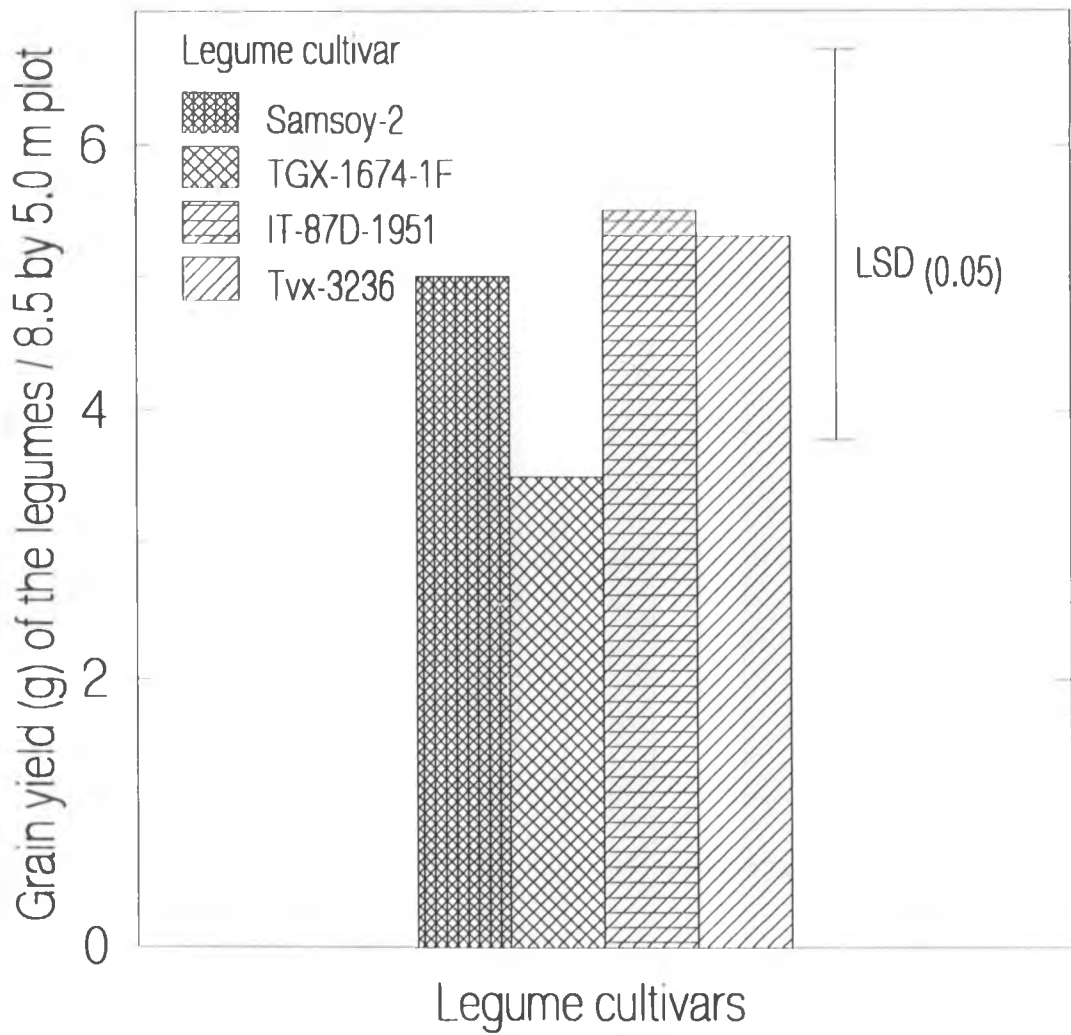
Fig. 1: Grain yield (g) of soybeans (Samsoy-2, TGX-1674-1F) and cowpea (IT-87D-1951 and Tvx-3236) grown during 1993 season.
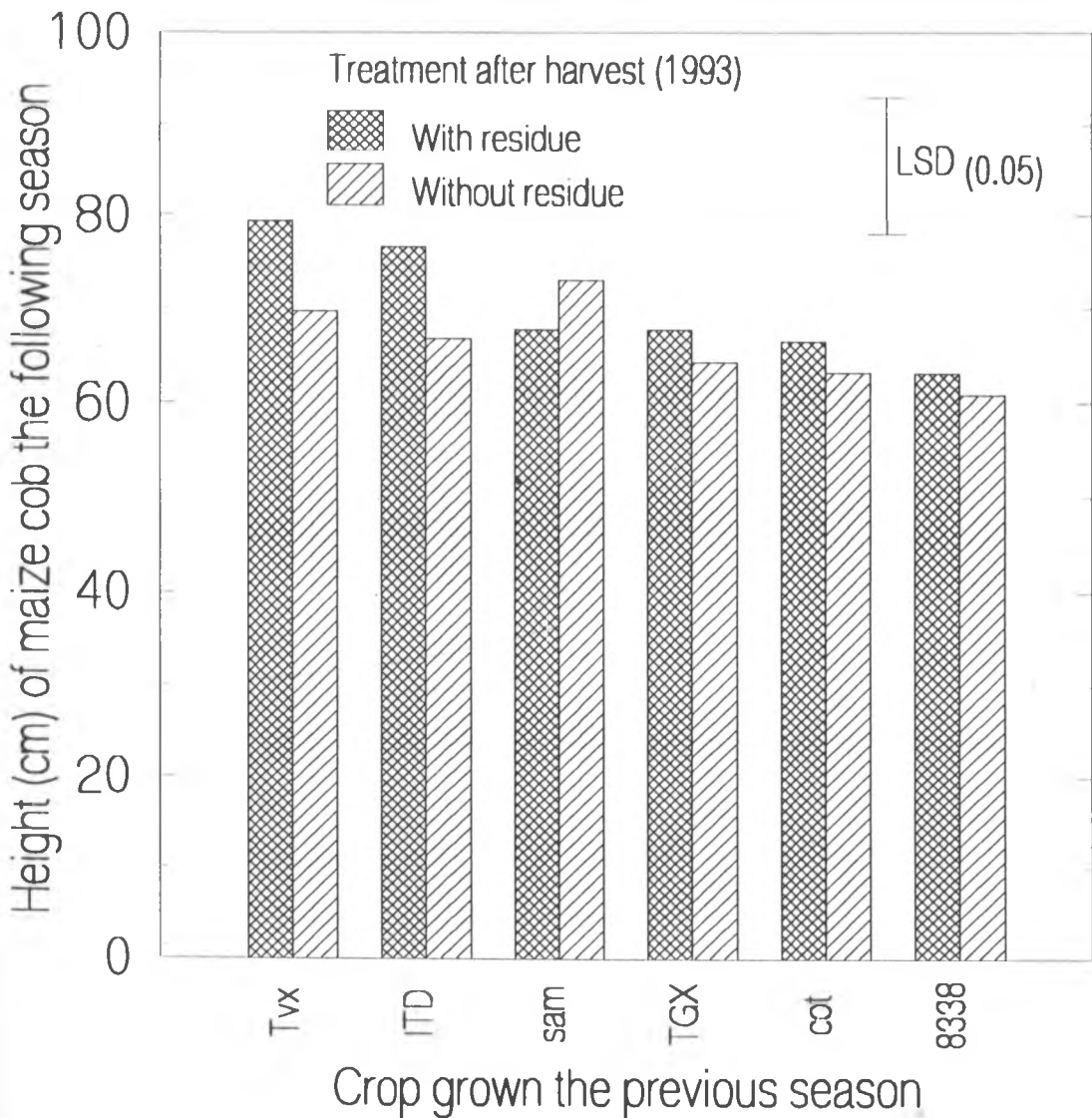
Fig. 2: Cob height (cm) of maize (1994) after planting cowpea (Tvx-3236, IT-87D-1951), soybean (Samsoy-2, TGX-1674-1F), cotton and maize (8338-1) the previous season (1993) with or without their residues.

Planting the two cowpea and soybean cultivars and cotton, the previous season, in soils infested with *Striga hermonthica* seeds significantly ameliorated the parasite symptoms on maize the following season (figure 3). Planting cowpea (Tvx-3236), the two soybean cultivars and cotton in 1993 season significantly reduced number of the parasite plants attached on each maize plant the following season. Addition of residues of the same cowpea cultivar was the most effective in reducing the number of the parasite plants on maize (figure 4). Planting cowpea (IT-87D-1951 and Tvx-3236) and Samsoy-2 and applying their residues prior to planting maize the following season significantly increased maize grain yield (figure 5).
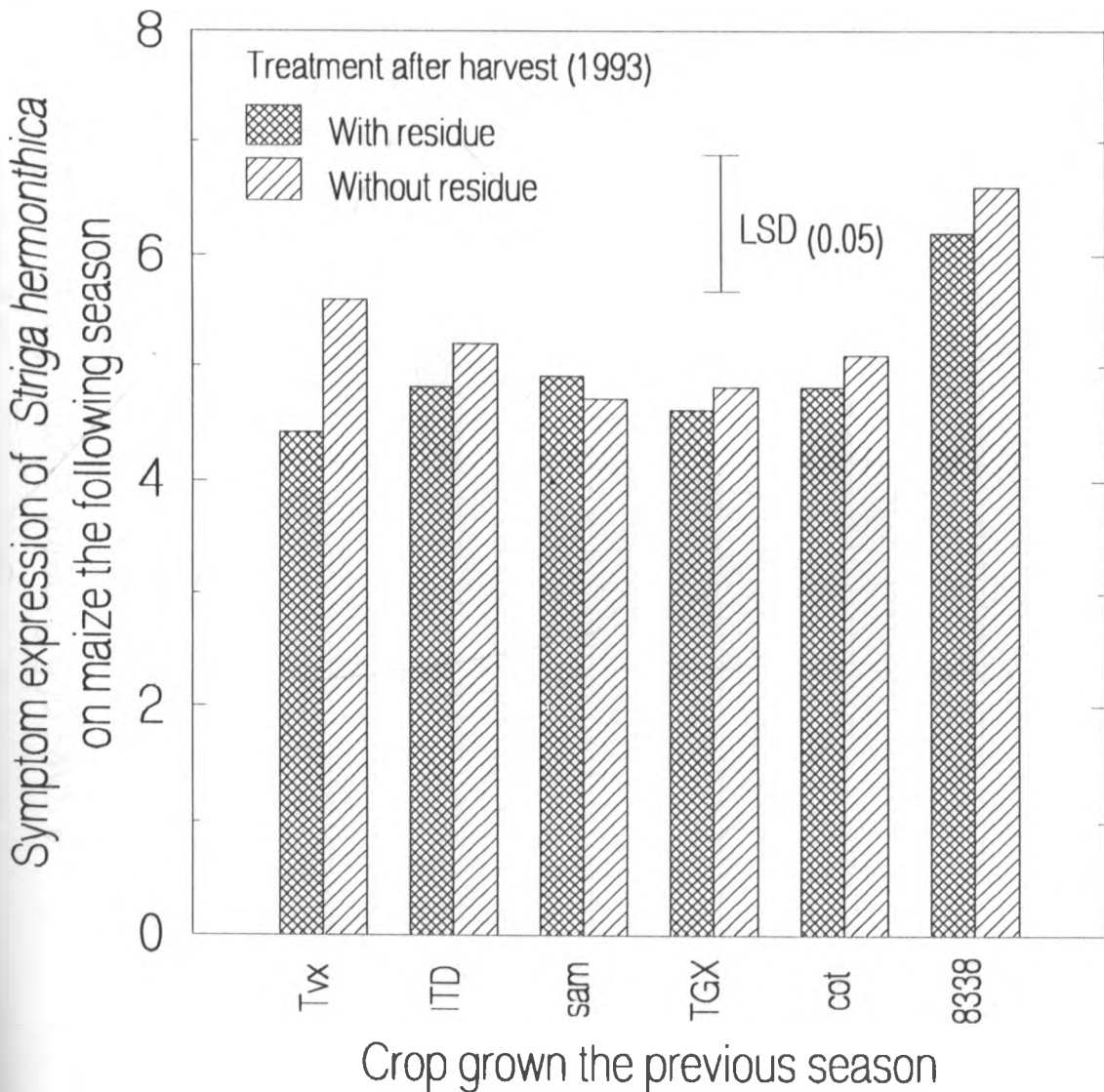
Fig. 3: Symptoms on maize (1994) after planting cowpea (Tvx-3236, IT-87D-1951), soybean (samsoy-2, TGX-1674-1F), cotton and maize (8338-1) the previous season (1993) with or without their residues.
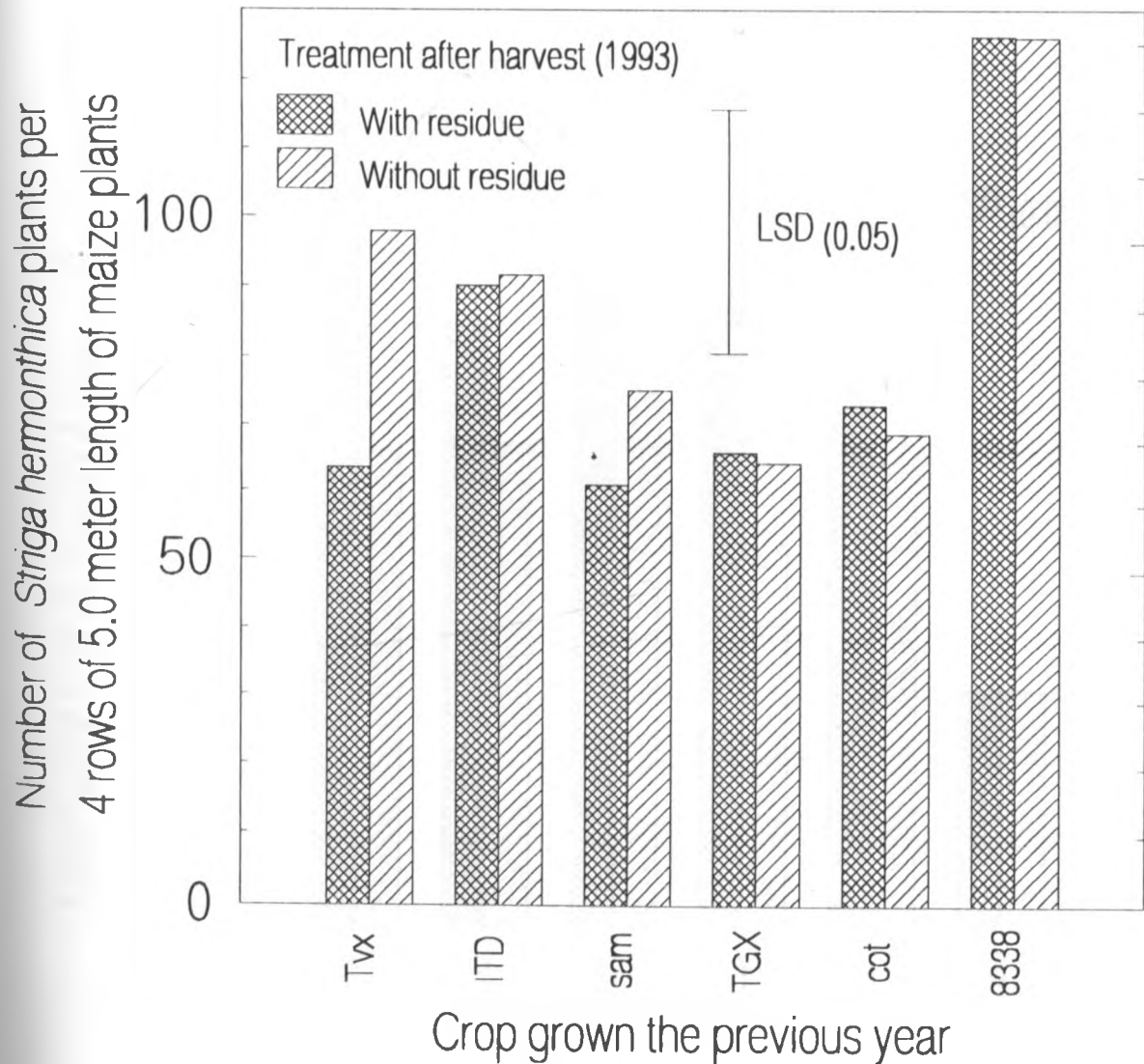
Fig. 4: Number of the parasites on maize after planting cowpea (Tvx-3236, IT-87D-1951), soybean (Samsoy-2, TGX-1674-1F), cotton and maize (8338-1) the previous season (1993) with or without their residues
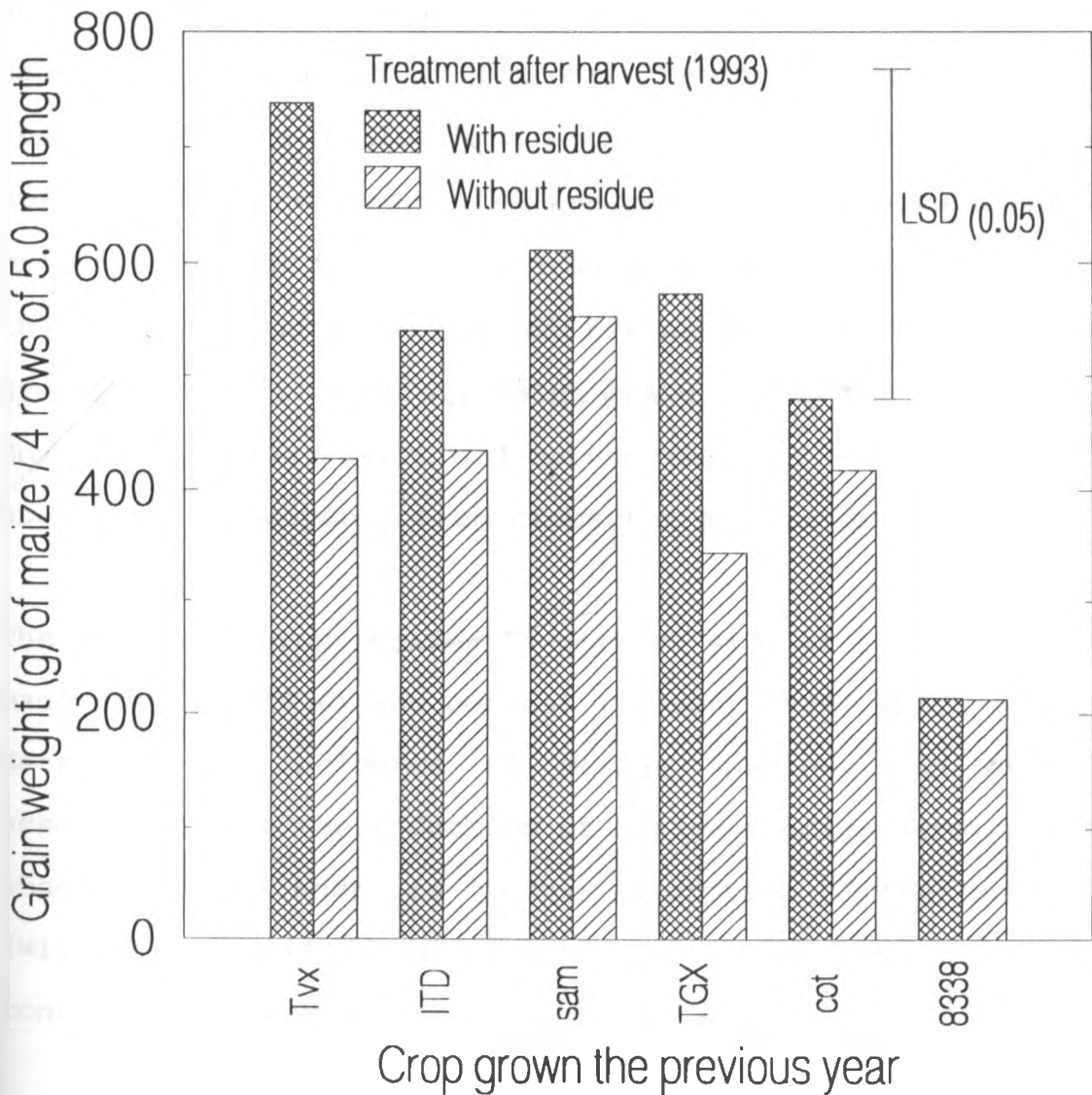
Fig. 5: Grain yield (g) of maize (1994) after planting cowpea (Tvx-3236, IT-87D-1951), soybean (Samsoy-2, TGX-1674-1F), cotton and maize (8338-1) the previous season (1993) with or without their residues

4:4 DISCUSSION

4:4:1 Effects of crop rotation on *S. hermonthica* on maize

Cowpea (Tvx-3236), soybean (TGX-1674-1F and Samsoy-2) and cotton (Abuja Local) may have caused the death of *S. hermonthica* seeds by stimulating them to germinate in the absence of a host the previous season. This explains the low parasite counts registered in each of these plots the following season. The presence of germination stimulants in the residues of the two cowpea cultivars and Samsoy-2 probably further induced suicidal germination of the parasite seeds thereby reducing their parasitism on maize and decreasing their symptoms on maize and increasing grain yields the following season. Soybean (Andrews, 1947) and cotton (Doggett, 1953) have also been suggested as suitable trap crops for *Striga hermonthica*.

The effect of crop residue is perhaps similar to that of farm-yard manure (Watt, 1936) and compost (Timson, 1939) which were noted to decrease the incidence of *Striga* species. The decrease was associated with the induction of *Striga* species germination in the absence of its host, or to the adsorption of the host stimulant (Wilson-Jones, 1953), or possibly to the increase in soil moisture content or microbial activity of the soil.

The reduction of the *S. hermonthica* parasitism on maize in 1994 after growing the legumes the previous year (1993) cannot be

attributed to their ability to fix nitrogen as cotton was equally effective. It has been reported that mineral fertilizers including nitrogen and farmyard manure did not significantly reduce *Striga hermonthica* infestation on maize (Smalling *et al*, 1991 and Osman *et al*, 1991). Osman *et al* (1991) reported that nitrogen seems to neutralize the harmful effects of the parasite without reducing the extend of parasitism. The effectiveness of soybean (Parkinson *et al*, 1988) and cotton (Bebawi and Michael, 1991) in reducing *S. hermonthica* parasitism when grown in rotation with maize.

5: GENERAL CONCLUSIONS AND RECOMMENDATIONS

From the objectives and the results of the experiments, optimal conditions for bioassay of *S. hermonthica* seeds in the laboratory were established. For maximum germination of the parasite seeds, they should be conditioned on 8 mm glass fiber disks in the dark at 28°C for 4 to 14 days. The optimal concentration of GR 24 was 10^{-5}.

In the bioassay of the parasite seeds with plant extracts or stimulants, the use of disc method of incubation is better than using sunken wells and is therefore recommended. Sunken wells are not suitable in testing germination of *S. hermonthica* seeds as the extracts tend to have inhibitory effects on the parasite seeds. Where sunken wells are used to test germination of the parasite seeds with GR 24, then germination counts should be taken after 48 hours of incubation of the parasite seeds with GR 24, in the dark, at 28°C. In the event of shortage of filter papers, bioassay test of the parasite seeds should be done as this has no influence on the outcome of the test. However, the plant extracts should be applied carefully to avoid washing away the parasite seeds from the filter disks.

Different cultivars of cowpea, cotton and soybeans have varying ability to stimulate germination of *S. hermonthica* seeds. It is therefore necessary to do laboratory screening of the cultivars before using them as trap crops for the parasite in the field. This

would save time and money as the laboratory tests are cheap and simple. Roots of 10 day old seedlings of cotton and cowpea are recommended to test stimulatory activities of the crops to *S. hermonthica* seeds.

Cowpea aqueous extracts to be used in bioassay of the parasite seeds should be diluted to a concentration of 6.3 mg plant tissue per milliliter of water. The extract can be stored at 7°C upto 20 days till required for the test. To detect germination of *S. hermonthica* seeds by dichloromethane soluble extracts from cotton and cowpea roots, the residues of the DCM extracts should be dissolved in concentrated dimethylsulfoxide (DMSO) and diluted to 0.5 and 1.0 percent respectively. However, for cotton leaves and cowpea stems dilution to 0.1 percent DMSO is recommended.

Aqueous extracts from roots of 10 day old plants of cotton and cowpea have the highest germination stimulation of *S. hermonthica* seeds. Stimulatory activity of the extracts from all parts of the plant decrease with age of the plant. Extracts from the seeds of these plants have no germination activity of the parasite seeds.

Cotton, cowpea and soybeans are trap crops for *S. hermonthica* and thus can be grown in rotation to control the parasite in maize. Upon harvesting these trap crops, their residues should be incorporated into the soil immediately. These residues contain the parasite seed germination stimulants, which would further deplete

the parasite seed reservoir from the soil by suicidal germination.

More work should be done to determine the optimal number of seasons required to rotate the trap crops with maize in order to achieve the best results. The contribution of their residues to soil fertility should be quantified. Thin Layer Chromatography results indicated that perhaps *S. hermonthica* seeds are stimulated to germinate by more than one compound in dichloromethane soluble extracts. This theory should be confirmed by performing the parasite bioassay tests of each compound scraped from every relative front on thin layer chromatography plate. The stimulatory compounds isolated from cowpea, cotton and soybean should also be identified through their structures and molecular weights. This would enable the synthesis of cheap analogs to induce suicidal germination of *S. hermonthica* seeds in infested farmers' fields. Emphasis should also be placed on identification and testing of the parasite seed germination inhibitors to reduce its parasitism on susceptible crops.

APPENDICES

Appendix 1: Monthly mean, maximum, and minimum mean air temperature at Abuja (1993 / 94)
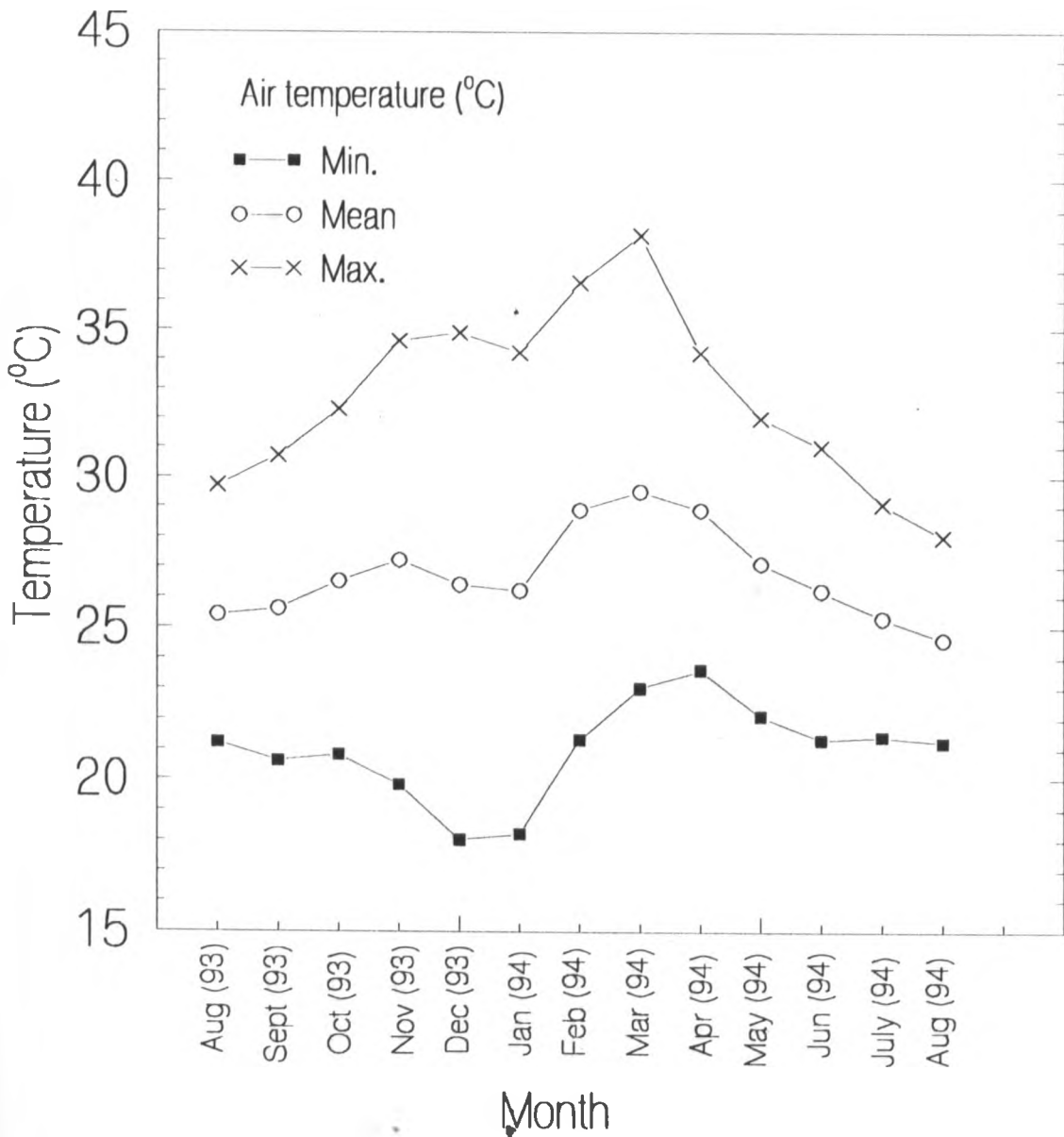
Appendix 2: Monthly mean, maximum, and minimum mean relative humidity at Abuja (1993 / 94)
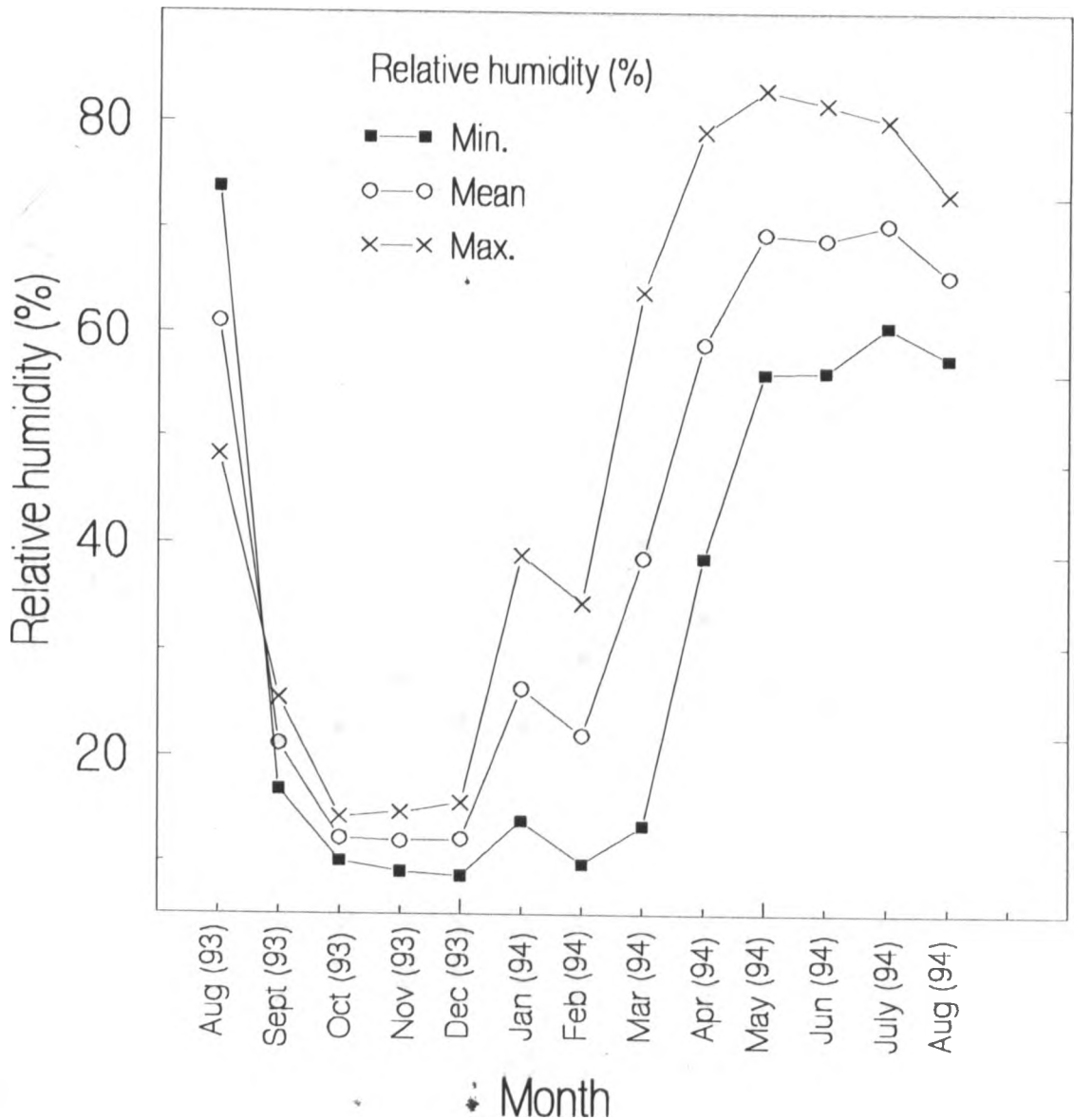
Appendix 3: Monthly mean, maximum, and minimum mean soil temperature at 10cm depth at Abuja (1993 / 94)
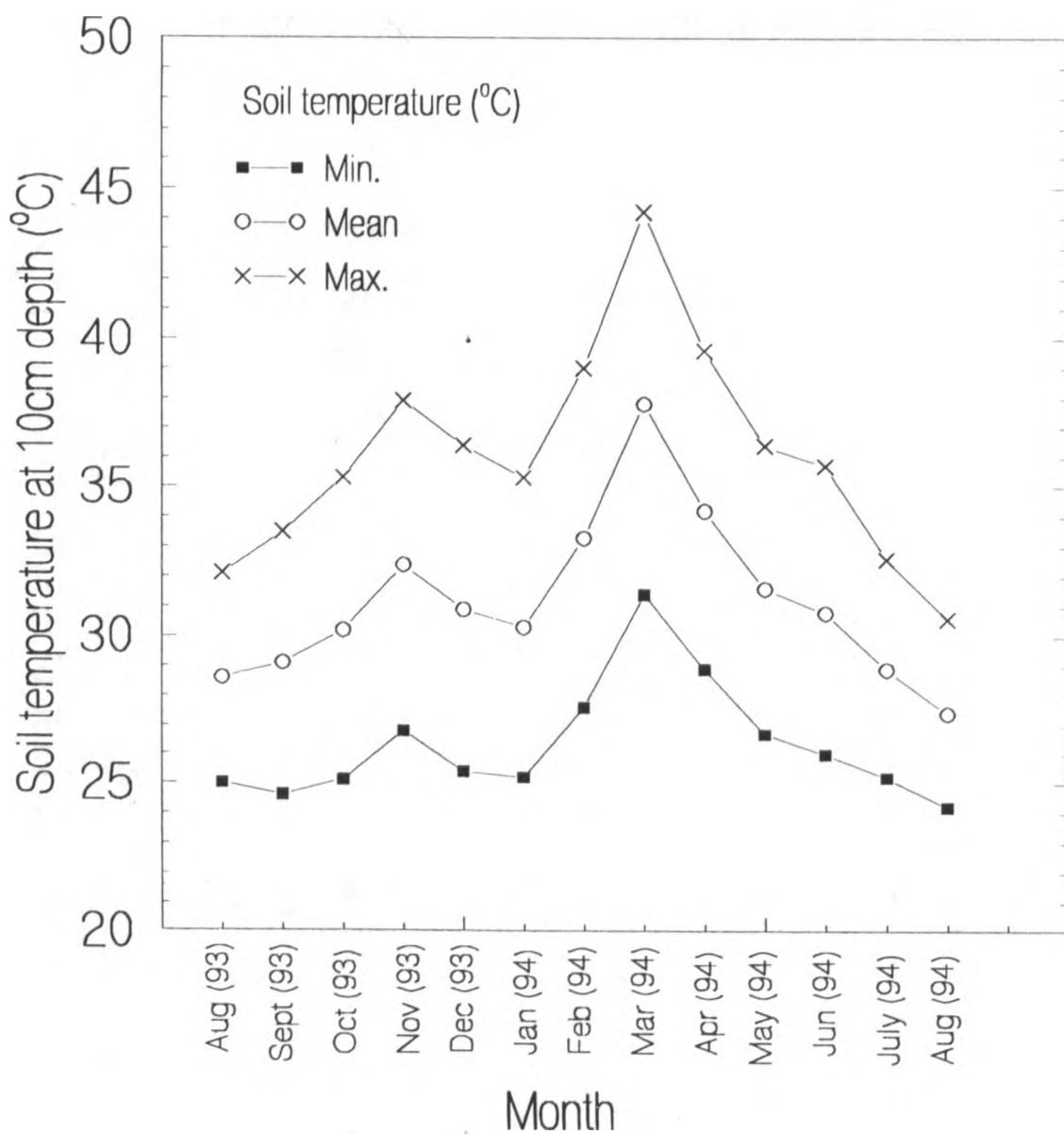
Appendix 4: Monthly mean, maximum, and minimum mean soil temperature at 20cm depth at Abuja (1993 / 94)
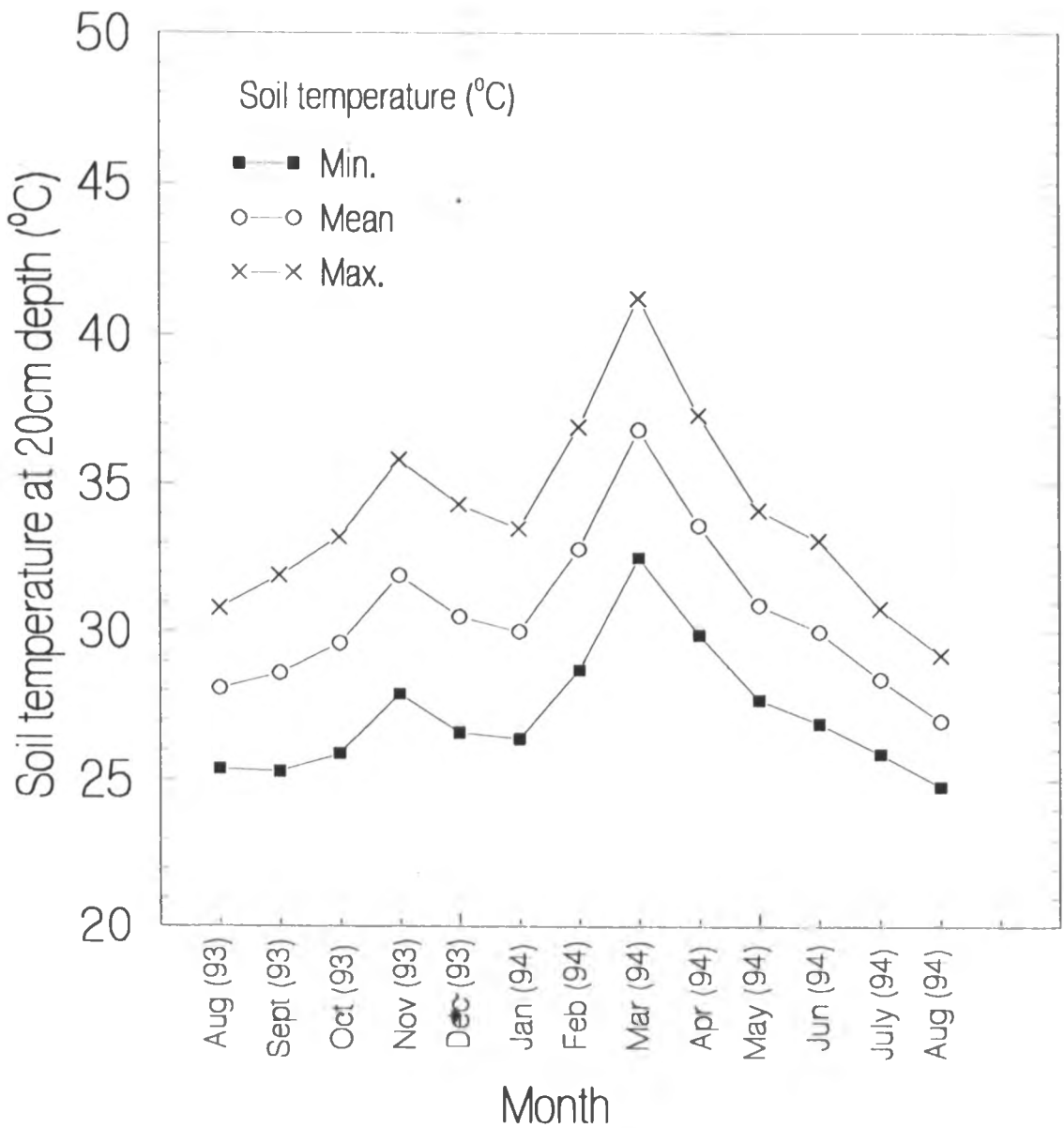
Appendix 5: Mean monthly rainfall at Abuja (1993 / 94)
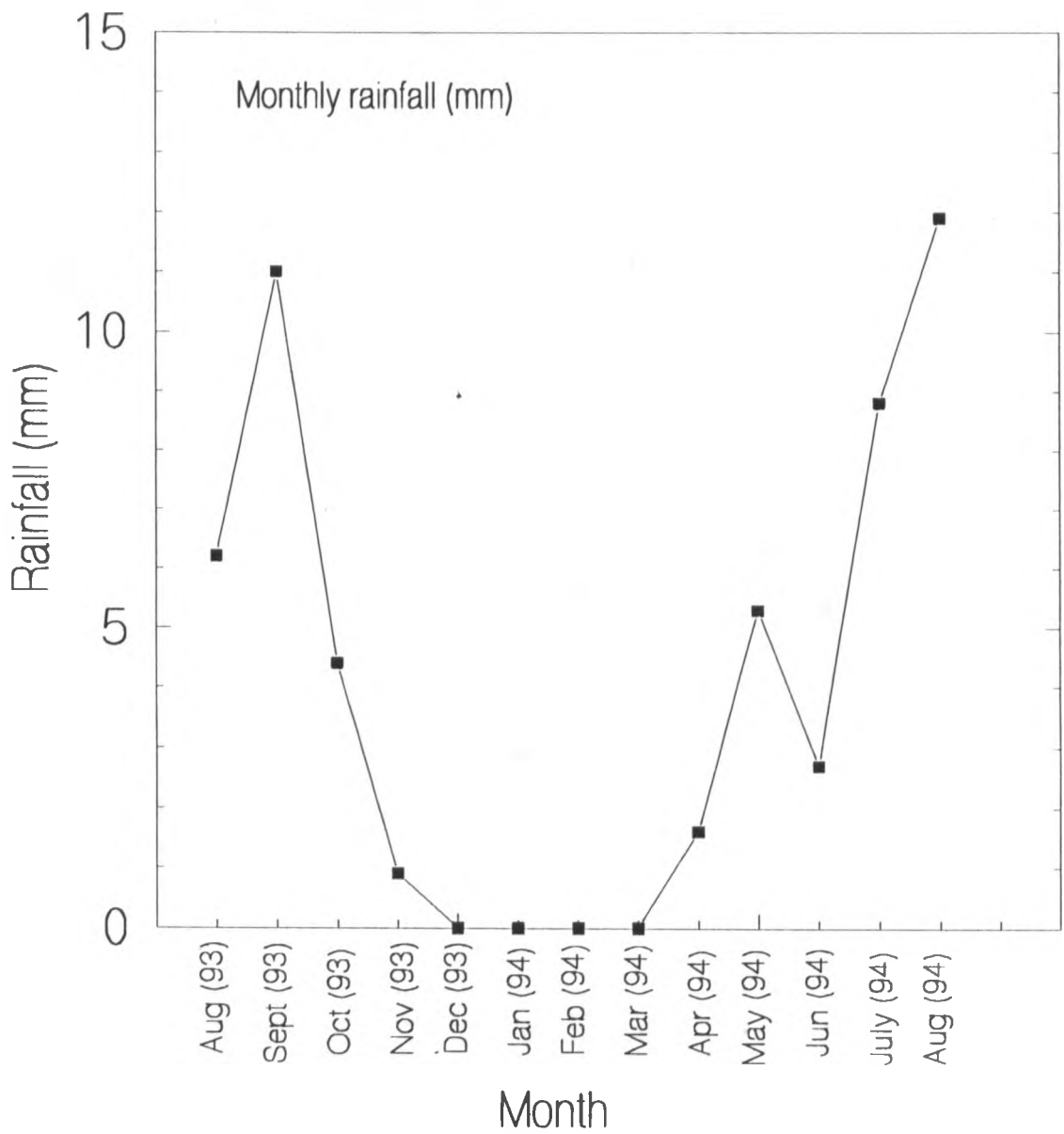
Appendix 6: Average monthly photosynthetically active radiation at Abuja during 1993 / 1994 season

Monthly photosynthetic active radiation
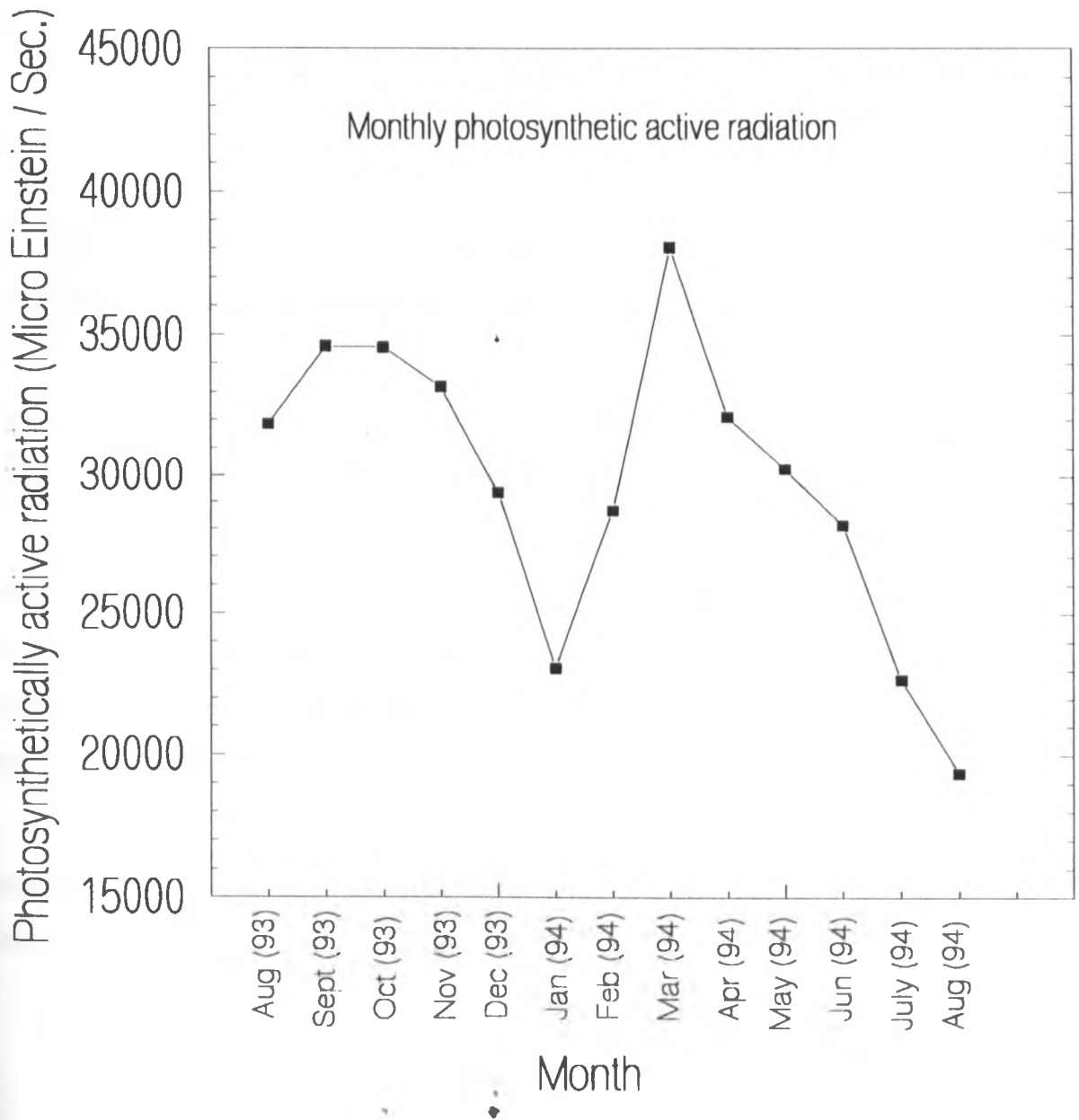
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